ACTA PHYSIOLOGICA LATINO AMERICANA

NUMERO DE HOMENAJE

AL

Profesor Alejandro Lipschutz

VOLUMEN 3

Nos. 2 y 3

1953

ACTA PHYSIOLOGICA LATINOAMERICANA

Editada por la Asociación Ciencia e Investigación

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Suscripción:

Cada tomo (cuatro números trimestrales) $ 50.— m/n. Exterior: 5 dólares

REPRESENTANTES:

Brasil: Editora Guanabara, Waissman-Koogan, Ltda.
Rua do Ouvidor, 132, Río de Janeiro.

EE. UU.: Stechert-Hafner Inc.
31 East 10th Street, New York 3, N. Y.

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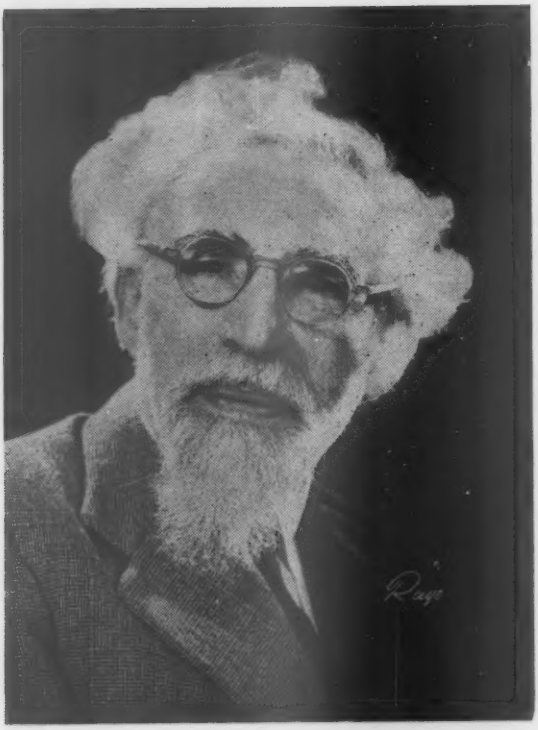
ACTA PHYSIOLOGICA LATINOAMERICANA

Vol. III - Nos. 2 y 3

1953

ASOCIACION CIENCIA E INVESTIGACION

Buenos Aires - Argentina



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Profesor Alejandro Lipschutz

EN SU 70 ANIVERSARIO,
POR SUS AMIGOS Y COLABORADORES.

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ALEJANDRO LIPSCHUTZ

LOS QUE cultivan las ciencias biológicas en la América latina tributan su homenaje de alto aprecio al Profesor ALEJANDRO LIPSCHUTZ, al cumplirse los 70 años de su fecunda existencia, por la valiosa obra científica de su escuela, por su papel de iniciador e impulsor de la fisiología y endocrinología experimentales y por su acción como maestro estimulante, cuya influencia ha traspasado las fronteras de Chile, su patria adoptiva, ha irradiado a toda la América y ha llegado a Europa y Norteamérica.

Los biólogos de todo el mundo conocen y respetan su obra y por eso, en numerosas ocasiones, ha sido invitado a dar conferencias en Inglaterra, Francia, Suiza, Portugal, Estados Unidos, España y Brasil y la mayoría de los países de lengua española, como huésped de las instituciones científicas oficiales o privadas más prestigiosas de dichos países.

La vida científica del Profesor Lipschutz se desarrolló en dos etapas: la europea y la americana. Nacido en Riga, el 28 de octubre de 1883, obtuvo su grado de Doctor en Medicina en la Universidad de Gottingen, en Alemania. Luego fué docente en Berna (1914-1919) y profesor titular de la Universidad de Dorpat (1919-1926). De 1908 a 1912 trabajó en Fisiología del sistema nervioso, con el Profesor Max Verworn y en metabolismo comparado. De 1914 a 1918 estudió los problemas sociales de la alimentación humana; de 1919 a 1920 la atrofia muscular y desde 1915 la endocrinología sexual.

Durante ese período publicó un libro sobre la Fisiología general de la muerte (en 1915) y uno de divulgación: "¿Por qué morimos?", que tuvo 14 ediciones en alemán (1914 a 1922) y se imprimió también en ruso, finlandés, estoniano, letón, español y portugués.

La etapa americana se inició en 1926, cuando fué llamado para organizar el Instituto de Fisiología de Concepción. Fué profesor de dicha materia y primer decano de la Facultad de Medicina de la Universidad de Concepción (Chile). Allí desarrolló una obra intensa, formó numerosos discípulos y fundó la Sociedad de Biología de Concepción y su Boletín.

En sus primeros trabajos se consagró al estudio de la función endocrina de las gonadas. Poco antes de venir a América había aparecido su clásico libro sobre la secreción interna de las glándulas sexuales, publicado en Suiza (1919), Inglaterra (1924) y España (1928). En Dorpat estudió principalmente la secreción interna del testículo y en Concepción la del ovario. Fruto de estos trabajos fué su libro sobre "Transplantación ovárica", editado en Madrid (1930) y Leipzig (1930), que expone descubrimientos fundamentales.

En el debatido problema de la interacción entre las hormonas sexuales demostró que puede observarse: 1º) un antagonismo frecuente en su acción directa sobre los órganos periféricos, lo cual había sido negado; 2º) un antagonismo sexual indirecto al través de la acción sobre la hipófisis, por ej.: atrofia testicular por los estrógenos que inhiben la secreción de gonadotrofina hipofisaria que mantiene el testículo; 3º) casos menos numerosos de sinergia.

Demostró la importancia de los factores extragonadales en la regulación cuantitativa de la función ovárica. Demostró la ley de la constancia numérica folicular, o sea que es constante el número total de folículos que maduran y ovulan en cada ciclo, ya existan uno o los dos ovarios. Esto se debe a la acción reguladora de la hipófisis y son también las gonadotrofinas hipofisarias las que rigen el desarrollo puberal de las gonadas.

El contenido y secreción de gonadotrofinas es distinto en la hipófisis de diversas especies. También es diversa en los dos sexos, o sea que hay una especificidad sexual extragonádica, pues la hipófisis es diferente en machos y hembras y esta diferencia es un carácter sexual secundario propio de cada sexo.

Para establecer la diferencia en la acción hipofisaria recurrió Lipschutz a métodos cuantitativos. Uno de ellos es el coeficiente de luteinización producido en el ovario por diversas gonadotrofinas. Comprobó que al asociar las hipófisis de diversas especies entre sí o con las de la orina, se completan o suman sus efectos.

Puede romperse el equilibrio normal recíproco que existe entre la hipófisis y el ovario. En ese caso, se observan trastornos de la regulación sexual o sea disrregulación ovárica. Esto se observa en diversas circunstancias: injerto ovárico o fragmentación ovárica. Si se fragmenta "in situ" el ovario de un cobayo y quedan fragmentos, después de poco tiempo se establece una hipersecreción estrogénica del ovario que la anterohipófisis no es capaz de moderar o frenar como en el estado normal. Hay prolongación de la acción estimulante folicular hipofisaria, sin que se equilibre con la acción luteínica. El hiperestrogenismo produce hipertrofia del endometrio, con proliferación atípica glandular y metaplasia epitelial del cuello; ese estado es semejante a la hipertrofia quístico glandular. Hay estimulación intensa del útero, vagina y mama. Tardíamente pueden producirse neoplasias del endometrio con caracteres de malignidad.

Otro caso de trastorno de regulación se produce al injertar ovarios en el bazo. El hígado metaboliza los estrógenos producidos por el ovario. Privada de la acción moderadora producida por los estrógenos, la hipófisis se desenfrena y aumenta su secreción de gonadotrofinas. El exceso de las mismas produce gran aumento de tamaño del injerto ovárico, primero con folículos hemorrágicos y luego con intensa proliferación de células luteínicas, que adquiere aspecto tumoral. Ha estudiado, utilizando la situación circulatoria tan especial del ovario intraesplénico en hembras castradas, la intervención de varias hormonas esteroides en los mecanismos que relacionan ovario e hipófisis anterior.

También estudió los reflejos que regulan la acción de la hipófisis sobre las glándulas sexuales o la mama.

Al separarse de la Universidad de Concepción, Lipschutz fundó en 1937 el Departamento de Medicina Experimental del Servicio Nacional de Salubridad, establecido en Santiago de Chile. Allí prosiguió con intensidad su obra de investigador y de maestro.

Desde 1935 se consagró su escuela al estudio del papel de las hormonas en la producción o prevención de los tumores. Fruto de su obra y su experiencia es el libro *Steroid Hormones and Tumors*, publicado en 1950.

Estudió principalmente la producción de fibromas subperitoneales en el cuy por acción de los estrógenos y sus ésteres. Comprobó el papel protector del hígado y la acción antitumoral de hormonas naturales (progesterona y desoxicorticosterona) o artificiales. El resultado del estudio sistemático de las condiciones cuantitativas y cronológicas bajo las cuales el estrógeno adquiere capacidad tumorígena, constituye una contribución de primera importancia y valor para la clínica humana Es la continuidad de la acción, durante un tiempo suficiente, de pequeñas cantidades de estrógeno la que transforma este estímulo fisiológico en inductor de un crecimiento anormal. De allí que es importante que sea cíclica la función del ovario como mecanismo de autodefensa antitumoral. En esa protección se combinan la ritmicidad de producción de los estrógenos con la acción, también periódica, de la progesterona, que impide la acción tumorígena de aquéllos.

También en ese Instituto se estudió: a) la producción de tumores uterinos por trastorno de la regulación hipofiso-ovárica, en la fragmentación del ovario; b) la formación de tumores adenomatosos de la *rete ovari* o de células luteínicas del injerto intraesplénico del ovario; c) la producción experimental de tumores de la región prostática por la acción prolongada de los estrógenos.

Estas investigaciones fundamentales han aclarado numerosos conocimientos básicos sobre tumorogénesis y antitumorogénesis y representan adelantos de la mayor importancia. Fueron realizadas en colaboración con sus discípulos, R. Iglesias, L. Vargas, E. Egaña, S. Bruzzone, A. Riesco, F. Fuenzalida, Elvira Mardones y con muchos otros jóvenes chilenos y extranjeros.

Diversas circuntancias mundiales despertaron en el Profesor Lipschutz el interés por problemas de Antropología. Algunos en el dominio científico, como ser el estudio de los grupos sanguíneos y la etnografía de los indios fueguinos. Otros relacionados con la historia, la sociología y la política, que expuso en sus libros sobre el Indoamericanismo y el problema racial en las Américas (1944). Se esfuerza por mejorar las condiciones de vida de los aborígenes y es uno de los miembros más activos del Instituto Indigenista de Chile.

Su obra científica le ha valido el respeto y aprecio en todo el mundo. Recibió el premio Charles L. Meyer de la Academia de Ciencias de los Estados Unidos (1944) por sus investigaciones sobre el cáncer. Es miembro honorario de numerosas sociedades de Biología y Endocrinología de América y Europa, profesor honorario de varias Facultades de Medicina de la América Latina, miembro correspondiente de prestigiosas sociedades científicas europeas. Forma parte del Comité científico de numerosas

revistas científicas. Es miembro Académico de la Facultad de Medicina de la Universidad de Chile.

El Profesor Lipschutz es un gran maestro que ha creado una escuela vigorosa y ha formado numerosos discípulos de alta calidad. Chile y la América latina le deben profunda gratitud por ello. Prueba de la atracción que ejerce es el hecho de que ha dirigido 18 tesis de Médico Cirujano en el Instituto de Fisiología de la Universidad de Concepción y 80 tesis en el Departamento de Medicina Experimental, en Santiago de Chile, desde 1926 hasta 1953.

Cuando habla transmite su entusiasmo y argumenta en forma convincente y estimulante. Sus conferencias han sido siempre apreciadas por su calidad y por su forma. Ha sido invitado a dictarlas en Academias, Sociedades y Universidades de Inglaterra, Francia, Suiza, España, Portugal, Estados Unidos, Canadá, Brasil y la mayoría de los países de América latina.

Este ilustre investigador, de gran cultura general, maestro que ha despertado el amor a la ciencia entre los jóvenes, organizador y director estimulante, ha realizado una obra científica valiosa, creado una escuela vigorosa, difundido conocimientos modernos y ha sido y es un ejemplo extraordinario de consagración abnegada al deber. Por eso y por su obra científica, llegado a los 70 años, aún fuerte y capaz, se ve rodeado por la admiración de sus colegas y el respeto y afecto de sus numerosos discípulos y amigos, que esperan aún mucho de su madurez.

BERNARDO A. HOUSSAY.

L'HIPERACTIVITÉ RÉACTIONNELLE DE L'OVAIRE RESTANT APRÈS HEMIOVARIECTOMIE

MAX ARON ET CLAUDE ARON

(Strasbourg)

En 1930, le Professeur A. Lipschütz, à qui est dédié le présent article, a montré que le nombre des follicules dans les ovaires obeit à une constance régie par des facteurs hormonaux, et a énoncé la "loi de constance folliculaire". Selon l'éminent sexuologue, l'ablation d'un ovaire, ou d'une partie du parenchyme ovarique, entraîne dans le parenchyme restant le développement d'un nombre supplémentaire de follicules qui rétablit l'équilibre compromis par l'exérèse.

Nos collaborateurs et nous-mêmes avons, au cours des dernières années, montré en différentes publications que l'ablation d'un ovaire provoque une modification immédiate de l'autre ovaire, caractérisée par l'hypertrophie et l'hyperplasie des follicules préexistants, et nous avons dénommé ce phénomène l' "hyperactivité réactionnelle de l'ovaire restant". Nous n'y trouvons pas de contradiction avec les faits décrits par A. Lipschütz. Mais, tandis que ceux-ci ont la signification d'une adaptation lente aux conditions créés par la suppression d'une partie du tissu ovarique, l'hyperactivité réactionnelle traduit une réponse subite à de telles conditions.

Au cours des lignes qui vont suivre, nous décrirons rapidement l'hyperactivité réactionnelle (H. R.), puis nous analyserons son mécanisme.

I. — CARACTÈRES DE L'HYPERACTIVITÉ RÉACTIONNELLE

L'H. R. présente un caractère cyclique. Au cours de certaines périodes, dites "positives", elle se produit chez le plus grand nombre des animaux auxquels on fait subir l'hémiovariectomie et, dans la majorité des cas, se montre forte et même intense. En d'autres périodes, dites "négatives", la castration unilatérale ne suscite, chez la plupart des opérés, aucune modification de l'ovaire restant, ou bien n'amène que des modifications discrètes. Les phases respectivement positives et négatives se succèdent sans régularité. Elles peuvent durer de quelques jours à quelques semaines. Elles ne présentent aucune relation avec le cycle oestral. Nous n'avons pas d'explication valable pour cette alternance.

Au cours des périodes positives, l'H. R. intervient rapidement après la castration unilatérale. Elle offre son maximum au bout de 48 h. Mais après un délai de 12 h, dans les cas de forte réaction, elle apparaît déjà évidente. Elle a pour signes des modifications accusées de la granulosa et de la thèque des follicules secondaires de toutes dimensions. Les follicules primaires sont atteints, non par l'hyperplasie, mais par l'hypertrophie. Les cellules folliculeuses et les cellules thécales augmentent de volume. Les mesures planimétriques montrent que leur surface peut augmenter du simple au double. Une activité cinétique notable s'installe à la fois dans la thèque et dans la granulosa, comparativement à l'ovaire-témoin. Le nombre des mitoses est d'autant plus élevé que l'hypertrophie cellulaire est plus forte. Il en résulte une croissance rapide des follicules.

C'est chez le Cobaye que nous avons d'abord constaté, puis étudié d'une manière approfondie l'H. R. dans l'ovaire restant. Il importait de démontrer que ce phénomène se produit également chez d'autres espèces. C'est ce que nous avons établi chez le Rat et chez le Lapin, où l'ovariectomie unilatérale entraîne des modifications de l'ovaire-restant qui offrent les mêmes caractères que chez le Cobaye.

II. — Déterminisme de l'hyperactivité réactionnelle

Selon A. Lipschütz, l'ablation d'un ovaire ou d'une partie plus importante du parenchyme ovarique total a pour effet une sécrétion accrue, par l'hypophyse, de gonadotrophine qui stimule le parenchyme ovarien résiduel.

L'H. R., telle que nous l'avons observée, dépend évidemment de la sécrétion gonadostimulante du lobe antérieur. L'ablation simultanée d'un ovaire et de l'hypophyse ne suscite plus l'hyperactivité de l'ovaire restant, qui involue dans les délais normaux, c'est-à-dire très rapidement, après l'opération.

Mais la question qui se pose est celle de connaître la cause de l'hypersécrétion de gonadostimuline qui intervient rapidement après l'ovariectomie unilatérale.

Sur la base des travaux antérieurs, on peut émettre l'hypothèse que cette hypersécrétion résulte du déficit hormonal (oestrogène) en rapport avec la suppression d'une partie du parenchyme ovarique.

Cette hypothèse ne cadre pas avec la série de faits ci-après:

1°) L'H. R. se produit chez le Cobaye et chez le Rat nouveau-nés, avec la même intensité que chez l'animal mûr, alors que l'ovaire ne contient que des follicules primaires ou de rares follicules secondaires à antrum à peine développé et que sa sécrétion interne, d'après l'état de repos des effecteurs, est nulle ou minime.

2°) L'administration de taux élevés d'oestrogène aux animaux avant l'ovariectomie ou lors de cette intervention n'inhibe pas l'H. R. lors des périodes positives (fig. 1).

3°) Si, au lieu d'un ovaire entier, on se contente d'enlever un fragment même minime d'ovaire, l'ovaire excisé et l'ovaire restant subissent les modifications caractéristiques de l'H. R., avec une intensité égale à celle de l'H. R. après hémiovariectomie.

4º) On provoque également l'H. R. sans supprimer une part quelconque du parenchyme ovarien, mais en se bornant à introduire dans un ovaire, au moyen d'un fin trocart, une substance inerte (chlorure de sodium), ou bien encore en sectionnant le mésovarium au ras de l'ovaire sans léser les vaisseaux principaux.

Il apparaît ainsi que l'H. R. résulte, non d'un déficit hormonal, mais de toute lésion de l'ovaire ou du mésovarium. Nous admettons donc que

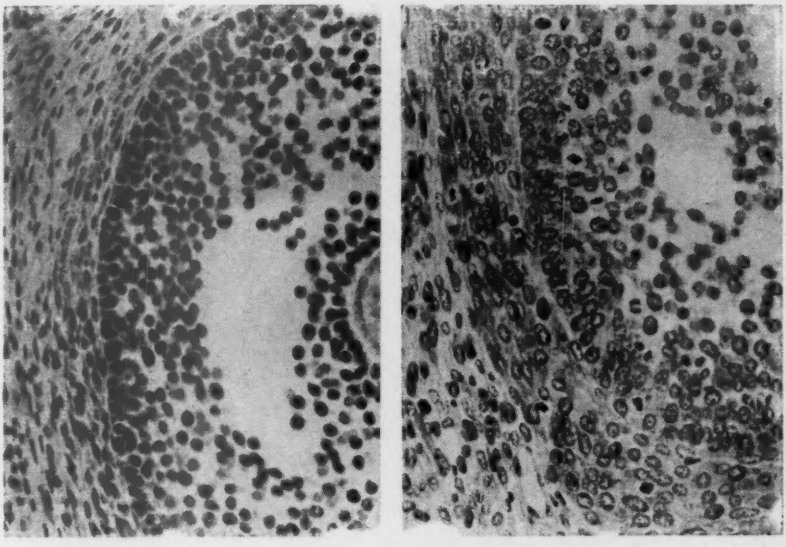

FIG. 1. — *Hyperactivité réactionnelle de l'ovaire-restant chez un cobaye de 130 g., préalablement traité par la folliculine. A gauche: follicule ovarique de l'ovaire-témoin extirpé après 4 injections de 500 U. I. de dipropionate d'oestradiol; l'activité cinétique thécale et granulosique est notable. A droite: follicule ovarique de l'ovaire-restant, prélevé 48 heures après l'hémicastration; l'on remarquera le degré extrême de l'hypertrophie et de l'hyperplasie thécale et granulosique.*

c'est l'excitation des nerfs efférents de l'ovaire qui est à l'origine de la reaction d'hyperactivité ovarienne. Tout se passe comme si cette excitation se transmettait à la préhypophyse, soit directement, soit par le relais des centres hypothalamiques, et provoquait une décharge de gonadostimuline. La rapidité avec laquelle se produit l'H. R. plaide en faveur de ce mécanisme. L'H. R. traduirait ainsi un *réflexe neuro-hypophysaire* à point de départ ovarien.

L'H. R. répond à une adaptation brusque de l'activité ovarienne à des facteurs de stimulation. Elle n'exclut pas l'avènement ultérieur d'une adaptation lente, correspondant au déterminisme invoqué par A. Lipschütz.

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THE ROLE OF OVARIAN SECRETIONS IN THE PATHOGENESIS OF CHEMICALLY INDUCED CANCERS

F. BIELSCHOWSKY

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FEW INVESTIGATORS have contributed as much as Professor A. Lipschutz (1950) to our knowledge of the role of ovarian secretion in normal and neoplastic growth of their target organs. It seems, therefore, appropriate to record in this issue dedicated to him some experiments designed to enquire into the role of ovarian hormones in the pathogenesis of experimental mammary cancers. Since Loeb (1919) discovered that ovariectomy performed early in life prevented the development of spontaneous cancers of the breast in mice it has become generally accepted that neoplastic growth does not develop in these organs in the absence of sex hormones. That holds true not only for the "spontaneous" mouse tumours but also for the chemically induced mammary cancers of rats. In this species the adrenals are unable to compensate for the loss of ovarian secretion, as in certain strains of mice, and therefore do not complicate the analysis of the hormonal factors involved in carcinogenesis of the mammary gland. It seemed of interest to enquire whether estrogens or progesterone is essential for the induction of tumors of the breast by the carcinogen 2-acetylaminofluorene (A.A.F.). When the ovaries of rats are autotransplanted into the tip of the tail the animals go into persistent estrous (Hernández, 1943). Histological study of such ovarian grafts reveals the absence of corpora lutea. Bielschowsky and Hall (1953) confirmed Hernandez findings and from their study of the ovarian transplants and of the accessory sex organs reached the conclusion that these grafts fail to secrete progesterone while estrogens are persistently secreted in sub-normal amounts.

This paper compares the incidence of mammary cancers induced by A. A. F. in normal and spayed rats with that found in animals bearing ovarian transplants in different sites.

Material and methods

Females from our colony of Wistar rats were used. Transplantation of the ovaries into the tail, lung or subcutaneous tissue was performed at the age of 4 weeks. When only one ovary was grafted the other was removed. Treatment with the carcinogen was started three weeks later. The animals received A. A. F. in their diet for a period of 13 weeks; during the first 9 weeks each rat received approximately 4 mg daily and 2 mg daily during the remaining period. The experiment was terminated 59 weeks after the commencement of the administration of the carcinogen. The majority of animals were killed when the presence of a tumour was discovered. However, some animals were not sacrificed after a breast tumour had made its appearance in order to test for the development of multiple mammary cancers.

Results

The females of the Dunedin strain of Wistar rats react to the oral administration of A. A. F. in a similar way to the albinos studied in

TABLE I

Incidence of breast cancers in intact and spayed rats and in rats bearing ovarian grafts.

	Nº of rats	Animals with breast cancers
Intact	17	11
Spayed	13	0
"Tail-grafts"	11	5
"Lung-grafts"	11	6
"Subcutaneous grafts"	13	5

Sheffield. In both colonies more than 60 % of the intact animals developed carcinomata of the mammary gland when the rats were not older than two months at the time treatment with the carcinogen was started. As Table I shows none of the animals spayed at the age of 4 weeks developed a breast cancer whereas a fair number of such tumours developed in animals bearing an ovarian graft. Multiple breast tumours occurred with each type of graft just as in the group of rats with the ovaries left in situ. The earliest cancer appeared during the 13th week of the experiment in an animal bearing a lung graft and the latest during the 59th week in a rat with the ovary transplanted into the axilla. In the intact females all of the eleven breast tumours appeared between the 19th and 35th week whereas in the grafted animals some of the tumours appeared considerably later.

Vaginal smears taken at regular intervals from rats with an ovary transplanted into the lung or the subcutaneous tissue revealed the presence of regular 4-5 day cycles throughout the experiment in the former and in the latter for the first 8 months. Later on some of these animals went

into persistent estrous and others into anestrous. The smears taken from rats bearing a tail graft consisted of cornified cells with an occasional insignificant admixture of a few leucocytes. Only in one of these animals the secretory activity of the graft seemed to decline towards the end of the experiment.

The ovarian grafts varied considerably as to their size and morphology. The majority of those transplanted into the lung or the subcutaneous tissue weighed 40-70 mg and contained multiple corpora lutea while the tail grafts were considerably smaller (20 mg or less) and were free of these structures.

Discussion

The number of animals used is too small to allow a statistical evaluation; however, the results suggest that, omitting the spayed animals, the incidence of mammary cancers induced in the different groups is of the same order of magnitude. One can therefore conclude that carcinogenesis can take place in the mammary glands of rats in the absence of a secreting corpus luteum. Since rats spayed prior to the administration of A. A. F. do not develop mammary cancers, adrenal secretions do not provide enough of progesterone and estrogen to allow neoplastic growth to take place and are therefore probably of little importance in the pathogenesis of these rat tumours. In an earlier publication (Bielschowsky and Hall, 1953) it has been pointed out that the amounts of estrogens secreted by the "tail grafts" are below those of the normal ovary since they fail to normalise the pituitaries which were found to be nearly as rich in gonadotrophins as the pituitaries of spayed animals. It seems therefore that ovaries persistently secreting sub-normal quantities of estrogen are nearly as effective as far as tumour development in the mammary gland is concerned as ovaries in situ. These results are not in conflict with those reported by Cantarow, Stasney and Paschkis (1948). These authors raised the incidence of mammary cancers induced by A. A. F. from 30 to 85 % by repeated injections of progesterone into intact animals, but failed to obtain breast tumours by this treatment in ovariectomized rats. Considering their results together with those reported in this paper it appears that the estrogens are the ovarian hormones essential for neoplasia in the mammary glands.

Summary

Mammary cancers have been obtained in nearly as high an incidence in rats bearing ovarian grafts as in intact animals. Such tumours developed whether the graft formed corpora lutea or not.

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ALGUNAS REACCIONES HISTOQUÍMICAS Y FUNCIONALES DE LOS MASTOCITOS

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EHRLICH (1879) descubrió los mastocitos (M.C.) reconociendo sus dos propiedades todavía hoy más notables: a) su afinidad para los colorantes básicos; b) su metacromasia. Luego se fueron agregando otras características morfológicas, histoquímicas y funcionales y se interpretó en términos bioquímicos el segundo de los hechos descubiertos por Ehrlich: la metacromasia.

Jorpes y colaboradores (Holmgren y Wilander, 1937; Jorpes, 1939); identificaron la metacromasia de los gránulos de los MC., con la que ofrece "in vitro" la heparina y emitieron la hipótesis de que las granulaciones de los mastocitos contenían heparina. Trabajos posteriores parecieron, en general, confirmar la hipótesis de Jorpes, y aún cuando algunos autores no se mostraron convencidos, no se describieron hechos fundamentales que invalidaran dicha hipótesis.

Poco se ha agregado, desde el punto de vista histoquímico, a lo anterior. La comprobación de la presencia de fosfatasa alcalina y de citocromo-oxidasa en las granulaciones de los MC, (Noback y Montagna, 1946) y la presencia, puesta en duda por algunos autores (Lillie, 1950; Compton, 1952), de una reacción de Schiff positiva en los gránulos, previa oxidación con ácido peryódico (técnica de Hotchkiss, 1948).

En el conocimiento de la función de estas células se ha adelantado poco. Pappenheim (1909) reconocía la completa ignorancia en que se estaba sobre este punto. Michels (1938) refiere en su monografía una serie de hipótesis, pero todas sin serio fundamento experimental. Aparte de la supuesta secreción de heparina, debemos mencionar los poco convincentes argumentos de Asboe-Hansen (1950 a.b.c.), que pretende que ellas forman la substancia fundamental del tejido conjuntivo. De mayor interés son las investigaciones tendiendo a comprobar la relación de los MC. con los esteroides suprarrenales. Ha sido comprobada por numerosos autores la acción de la cortisona sobre el tejido conjuntivo, en general, y la formación de granulomas en particular. Con el mismo criterio se buscó su acción sobre los MC. (Cavallero y Braccini, 1951; Asboe-Hansen, 1952; Bloom, 1952).

En este trabajo nos proponemos exponer algunas observaciones realizadas en nuestro laboratorio, sobre las reacciones histoquímicas de los gránulos de los MC. y la acción de la cortisona sobre esas células.

Reacciones histoquímicas de las granulaciones de los mastocitos

Un colorante que posee una gran afinidad para las granulaciones de los MC. es la fucsina básica. La hemos usado tal como la recomienda Gallego, o sea en forma de fucsina fenicada de Ziehl, diluída en agua acética y luego virofijada en agua aceto-formólica. En esta forma las granulaciones de las MC. aparecen intensa y regularmente coloreadas en rojo, con un tinto ortocromático. Es el método, a juicio nuestro, más favorable para la demostración de tales elementos. Con la idea de analizar la razón de esta coloración, hemos hecho ensayos usando la técnica de Coujard (1943) para el análisis histoquímico de sustancias puras. Escribiendo, según dicha técnica, sobre un porta con gelatina, y con gelatina conteniendo heparina en solución al 1/2000, comprobamos que, en láminas sin fijar y en aquellas fijadas por el alcohol absoluto, la gelatina no se tiñe por la fucsina de Gallego, en cambio lo hace la heparina, tomando el color rojo característico. Puede admitirse, en consecuencia, que la afinidad de la fucsina básica por las granulaciones de los MC. se deba a su contenido en heparina.

En una serie de estudios sobre los elementos argentafines de distintos órganos realizados en nuestro Departamento (Acosta Ferreira, 1952; Grasso, 1953), se pudo comprobar, repetidamente, que en ciertas condiciones las granulaciones de los MC. presentan el fenómeno de la argentafinidad (1). Es la técnica de Río Hortega para elementos argentafines, la que ofrece resultados más satisfactorios. Con ella las granulaciones aparecen gruesas, ya sea aisladas o más frecuentemente aglutinadas y compactas (Figs. 1 y 2). Si luego de la impregnación argéntica se sobrecolorean las secciones con la fucsina de Gallego, aparecen los MC. bajo tres formas distintas: a) algunas con todas sus granulaciones negras, teñidas exclusivamente por la plata; b) otras con sus granulaciones rojas, teñidas exclusivamente por la fucsina; y c) un tipo intermediario en que coexisten granulaciones rojas y negras (Grasso, 1953). Grasso admite que se trata de etapas evolutivas de un proceso que empieza por granulaciones fucsinófilas (no argentafines) pequeñas y culminan en los grandes gránulos argentafines.

La posibilidad de que la reacción argentafín sea atribuible a la heparina está abonada por el hecho, reiteradamente observado, de que soluciones de heparina hasta 1×10^{-4} reducen las sales argénticas cuando se las estudia por el procedimiento de Coujard.

Tanto las granulaciones de los MC. como las marcas sobre portas hechas con heparina, son negativas a la diazorreacción y a la cromoreacción.

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(1) Es decir que reducen las sales argénticas. Las argentofilia es, al contrario, la propiedad de retener selectivamente las sales argénticas, que son secundariamente reducidas por substancias químicas diversas (pirogalol, hidroquinona, formol, etc.).

Hemos aplicado las técnicas de coloración con el reactivo de Schiff, previa oxidación con ácido peryódico, al estudio de las granulaciones de los MC. Diversos autores han llegado a diferentes resultados; algunos (Leblond, 1950; Montgomery, 1953), han obtenido coloración de los gránulos de los MC. por la técnica de Hotchkiss. Otros investigadores (Lillie, 1950; Compton, 1952), han encontrado resultados discordantes. Lillie (1950), en un cuidadoso estudio, sólo encontró algunos granos fucsinófilos, luego de oxidación por ácido peryódico, en raros MC. Davies (1952) estudiando muestras de hialuronato y de heparina por una técnica similar a la de Coujard, comprobó que la heparina daba intensa reacción con el proceder de Hotchkiss.

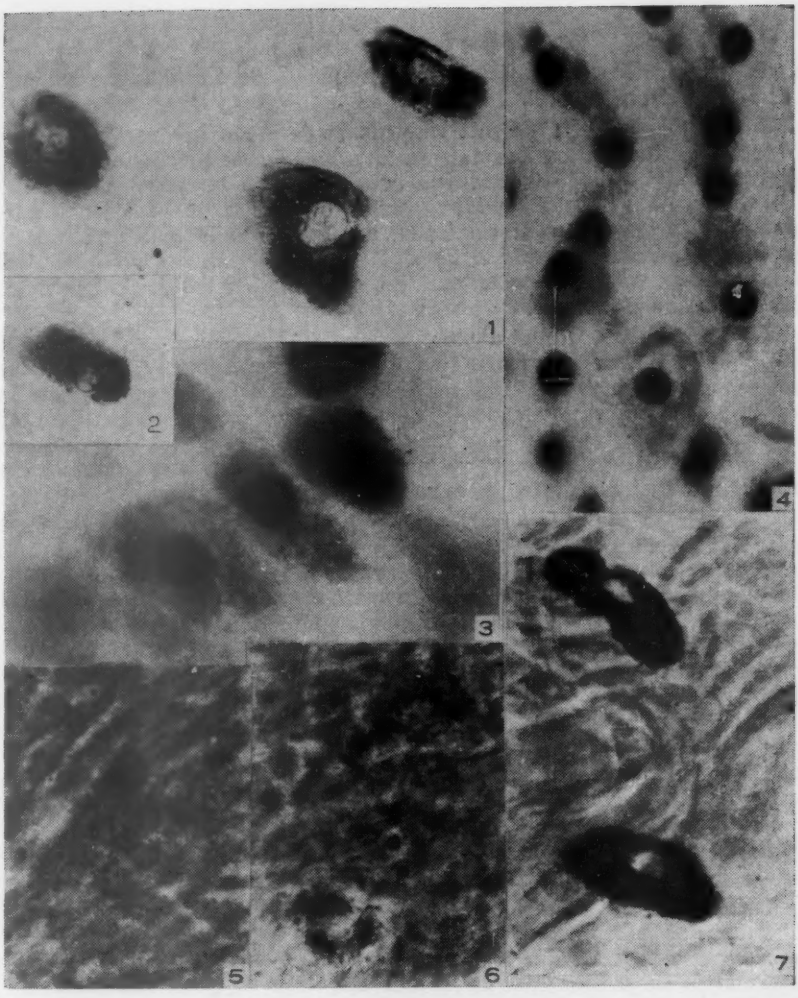
Por nuestra parte hemos comprobado que los MC. aparecen con cierta frecuencia teñidos difusamente por la fucsina. Sin embargo, esta tinción se observa también en los controles que no han sido oxidados por el ácido peryódico, lo que nos lleva a concluir que se trata de una reacción plasmal, o, más probablemente, de una coloración por cantidades muy pequeñas de fucsina no reducida, que permanecen en el reactivo de Schiff, o que difunden de regiones vecinas fuertemente coloreadas. Estas pequeñas cantidades de fucsina, que no coloran ningún elemento del tejido, se fijan, sin embargo, sobre las granulaciones de los MC. por su gran afinidad por el colorante.

Acción de la cortisona sobre los mastocitos

Es un hecho repetidamente comprobado que la cortisona tiene una acción neta sobre el tejido conjuntivo en general y sobre el proceso de cicatrización y de formación de las granulomas en especial. Cavallero y Bracini (1951), estudiando la acción de la cortisona sobre el tejido conjuntivo, hallaron, entre otros hechos, que la cortisona produce una desgranulación de los mastocitos. Asboe-Hansen (1952), comprueba esa acción de la cortisona sobre los MC, agregando que a la desgranulación se acompaña un proceso de vacuolización citoplasmática, con reducción del número de MC. Bloom (1952), comprobó la desaparición de un tumor maligno a MC., espontáneo, del perro, por inyecciones de cortisona. Los MC. degeneraban presentando vacuolización citoplasmática, aglutinación de los gránulos, pérdida de su colorabilidad y borramiento del límite celular con dispersión de los gránulos al exterior. Al contrario Schoch y Glick (1953), no hallaron ningún cambio en los MC., después de Stress, o inyecciones de ACTH y cortisona.

Todos estos autores emplearon la cortisona en inyecciones subcutáneas, comprobando la acción general de la hormona. Quedaba por resolver si su efecto era directo sobre los MC., o si actuaba por un mecanismo indirecto. Para analizar este punto, realizamos dos tipos de experimentos. En primer lugar (Buño y Poletti, 1953), hemos estudiado la acción local de la cortisona inyectada en el tejido celular subcutáneo de la rata. De un lado del animal, en una zona limitada, inyectamos la cortisona; del otro lado, simétrico, se inyectaba un volumen igual del vehículo, pero sin la hormona. Usamos una suspensión de acetato de cortisona * en una

* Agradecemos a Merck & Co. Inc., Rahaway N. J. que nos han cedido generosamente el acetato de cortisona empleado en estos estudios.



FIGS. 1 y 2. — *Mastocitos con granulaciones argentafines. Técnica de Río Hortega. (1): En el tejido conjuntivo de la próstata de la rata. (2): En la sub-mucosa del estómago de la rata.*

FIG. 3. — *Tejido conjuntivo subcutáneo de rata inyectada localmente con cortisona 4 horas antes. MC. en diversos grados de desgranulación y vacuolización.*

FIG. 4. — *Tejido conjuntivo subcutáneo de rata inyectada localmente con cortisona 4 horas antes. MC. en las últimas etapas de desgranulación.*

FIG. 5. — *MC. tratados "in vitro" con cortisona. Después de 2 horas de permanencia en la cortisona. Coloración supravital con azul de toluidina. Extrema desgranulación de los elementos. Observación con contraste de fases.*

FIG. 6. — *MC. tratados "in vitro' con cortisona. Después de una hora de permanencia en la cortisona. Coloración supravital con azul de toluidina. Aglutinación de los gránulos y desgranulación de los elementos. Observación con contraste de fases.*

FIG. 7. — *MC. coloreadas supravitalmente con azul de toluidina (control). Observación con contraste de fases.*

mezcla de solución salina fisiológica, con 20 % de alcohol, que contenía 20 mg de cortisona por ml.

Se inyectaba 0.5 ml de esta suspensión de modo que recibían 10 mg de cortisona. Tres horas después de la inyección de cortisona se inyectó una suspensión de litio-carmín (ya que se estudió también la acción de la cortisona sobre la coloidopexia) y una hora más tarde, es decir, 4 horas después de la inyección de cortisona, se hizo de cada lado una bola de edema, que se fijó en formol al 10 %. Se realizaron en ese material técnicas comunes, coloraciones con toluidina y la técnica del ácido peryódico-sulfofucsina de Hotchkiss.

Los resultados, limitándonos a los MC, fueron muy netos. Del lado inyectado con cortisona las granulaciones de los MC. aparecieron muy reducidas y en muchos casos habían desaparecido totalmente, quedando el citoplasma vacuolado, agranular, con su característica metacromasia, aunque también ésta muy reducida. Es frecuente el hallazgo de MC. cuyos límites celulares se vuelven imprecisos y como desgastados. Estas modificaciones no se comprobaron del lado testigo.

La segunda serie de experimentos para comprobar la acción directa de la cortisona sobre los MC., la realizamos "in vitro". Para esto utilizamos también ratas blancas, a las cuales hacíamos biopsias del tejido celular subcutáneo o del epiplón. Se retiraba un pequeño trozo de tejido conjuntivo que se recibía en líquido de Ringer aséptico. Este trozo se dividía en dos partes permaneciendo una de ellas, que servía como control, en líquido de Ringer; el otro trozo era llevado a una suspensión de acetato de cortisona en Ringer conteniendo 2 mg de cortisona por ml. Ambas eran mantenidas a 37°. A los 15 minutos, 30 minutos, 1 hora, 2 horas y 4 horas, se retiraban pequeños trozos, tanto del control como del tratado con cortisona, que se extendían sobre láminas porta-objetos, con una gota de una solución de azul de toluidina en líquido de Ringer. Se le colocaba un cubre-objeto que era cuidadosamente bordeado con vaselina. La observación se realizó siempre con contraste de fases y sobre platina del microscopio mantenida a 38°.

En esta forma se comprueba que solamente las granulaciones de los MC. toman el color en un tinte metacromático rojo violáceo. Los núcleos permanecen perfectamente incoloros por varias horas. La coloración nuclear, en cualquier elemento, se consideraba como signo de muerte celular y se desechaba el experimento.

En estas condiciones pudimos comprobar una neta acción de la cortisona sobre los MC. Esta acción comienza a la media hora alcanzando su máximo vigor entre la hora y las 2 horas de tratamiento.

Los fenómenos observados fueron: a) aglutinación de los gránulos formando grumos a veces tan grandes como el núcleo; b) disolución y dispersión de los gránulos; c) desgranulación celular con pérdida de los límites celulares y aspecto desgastado del elemento; d) alteración de la coloración metacromática que toma un tinte más rosado, como desvanecido, en comparación con el rojo violáceo de los elementos normales; e) aparición del tinto metacromático en el citoplasma intergranular, (Figs. 5, 6 y 7).

De los experimentos anteriores podemos concluir que la cortisona actúa, en forma directa, sobre los MC.

Conclusiones

1 — La coloración ortocromática de las granulaciones de los MC. por la fucsina básica, puede ser atribuible a la heparina. Esta sustancia se tiñe "in vitro" por la fucsina básica, utilizando la técnica de Coujard.

2 — Las granulaciones de los MC. son argentafines. Esta propiedad puede ser atribuible a la heparina, ya que esta sustancia la presenta "in vitro", utilizando la técnica de Coujard.

3 — La cortisona actúa directamente sobre los MC., ya sea en inyecciones subcutáneas, ya sea en experimentos "in vitro". Esta acción se traduce histológicamente por desgranulación del elemento, dispersión de los gránulos y ocasionalmente formación de vacuolas.

Summary

Mast cells (M.C.) are well known, since Ehrlich, by their metachromatic staining with basic dyes. This fact has been interpreted by Jorpes *et al.* as due to the heparin content of the granules. Basic fuchsin stains also deeply the granules of mast cells, although orthochromatically. This may too be due to heparin, since heparin spots on a slide, (Coujard's test) are deeply stained by basic fuchsin.

M. C. granules are often argentaffin. In some cases all the granules of a single cell are argentaffin; in other cells only some granules are argentaffin, others being deeply stained by basic fuchsin but not argentaffin; finally other M. C. stain only with basic fuchsin. Heparin "in vitro", (Coujard's test) also reduces the silver salts.

Hotchkiss technique for demonstration of polysaccharides has been claimed positive in M.C. granules, (Leblond, 1950; Montgomery, 1953), or negative (Lillie, 1950; Compton, 1952). We have found that positive staining can be imputed to the diffusion of recoloured fuchsin.

Several workers have shown that cortisone injected in animals produces degranulation and vacuolation of M. C. In this paper we show that this fact is due to a direct action of the hormone on the cells. M.C. from the cortisone injected region showed degranulation, while those of the opposite region of the same animal, injected only with the suspension fluid, were normal. In other experiments we put small pieces of sub-cutaneous tissue of rat, in Ringer and in Ringer with cortisone. After different periods of time they were observed, vitally stained with toluidine blue, under the phase contrast microscope. After an hour of stay in Ringer-cortisone the M.C. showed intense degranulation, as compared with control.

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THE DISCOVERY OF SMOOTH MUSCLE IN THE UTERUS

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THE DISCOVERY that the contractile wall of the uterus is composed of smooth muscle tissue laid the foundations of a great structure of theoretic and clinical knowledge about the function of that important organ. Wishing to know to whom honor should be given for this discovery, the author turned to the textbooks of medical history without obtaining an answer. A search of the original literature was then made, revealing a story that may appropriately be printed in this volume dedicated to Professor Alexander Lipschütz, himself a distinguished maker of discoveries about the reproductive system.

Long before the era of microscopic anatomy began, the uterus was rightly thought to be a muscular organ. There was ample direct evidence that the pregnant human uterus is able to undergo strong contractions, and from the time of Vesalius (1543) or earlier, anatomists knew that it is composed of bundles of parallel fibers (using the word *fiber* in the macroscopic sense) as are the voluntary muscles. The *Elementa Physiologiae* of Albrecht von Haller (volume VII, 1778) gives a summary of opinion on this subject which tells us concisely how much was known before the microscope was applied to the problem. Haller states that the uteri of quadrupeds are obviously muscular, "almost like the esophagus", but the human uterus appears at first sight to be different, being composed of a peculiar dense succulent tissue. He then quotes numerous authors whose observations suggest that this human uterine substance, in spite of its peculiarity, is muscular in nature; and speaking for himself says "De nomine *musculi* ego quidem nolim pugnare; sufficit extra omne dubium esse, in utero humano fibras musculosas, irritabiles reperiri, quarum aliae latae ad se invicem adducant transversae: & aliae cervicem ad fundum adtrahant, uterum reddant breviorem".

It must be added that this confident assertion of the muscular nature of the human uterus by Haller and the earlier observers he quotes, was based upon the pregnant uterus, in which the hypertrophied muscle tis-

sue forms coarse bundles, resembling the fascicles of the voluntary muscles.

When histology began to develop as a major science, about 1830, the muscular tissues like everything else in the body were subjected to microscopic examination, and before long it was found that muscle fibers exist in two forms, cross-striated and unstriated, the former occurring in voluntary muscles, the latter in certain viscera. The history of this period of exploration and discrimination, up to about 1840, is given by Henle in his *Allgemeine Anatomie*, in a way however which is not easily understood in present day terms, because in 1841 he was writing without full knowledge of the great advences which were being made by Sir William Bowman of London, whose now famous paper on the structure of voluntary muscle had been published in 1840. The art of isolating the individual elements (*i. e.* the compound primitive fibers of striated muscle, and the cells of smooth muscle) so as to permit clear observation, had not yet been developed. The cross-striations of voluntary muscle, seen in the 17th century by Leeuwenhoek, were still escaping the notice of many observers, in spite of their improved microscopes, and the question whether the two kinds of muscle are truly distinct types was not yet conclusively answered.

Bowman's article of 1840, however, described and illustrated the voluntary muscular elements with such clarity that their distinctive nature was firmly established. At the same time the characteristic cells of smooth muscle were also being recognized, and it too had to be accepted as a distinct tissue. Henle gives credit to Schwann, Valentin, Lauth, and R. Wagner for pioneering observations of these cells made between 1833 and 1839. Henle himself in his book of 1841 gives a clear description and good illustrations of smooth muscle fibers, which he had found in the gastro-intestinal canal; the excretory ducts of the salivary glands, pancreas and liver; the urinary bladder, vas deferens and seminal vesicles; and the trachea and bronchial tree. On the basis of this distribution he was able to confirm the characterization, already made by Schwann, of smooth muscle as involuntary, in contrast to the voluntary striated muscle.

Although Henle did not formally include the uterus in his list of organs containing smooth muscle, he said that E. A. Lauth had in 1834 reported seeing muscle fibers in the human uterus. Lauth's communication to the Société d'Histoire Naturelle de Strasbourg indeed contains, so far as I know, the first statement of this fact based on microscopical evidence. His brief description, however, shows that he could not have clearly seen the individual muscle cells, and that he did not recognize that they were essentially different from voluntary muscle fibers. Henle also refers to a passage from Schwann's *Mikroskopische Untersuchungen*, 1839, which although it is neither precise nor comprehensive clearly reports the existence of smooth muscle fibers in the uterus. I quote Schwann's words from the English translation of 1847:

"The involuntary muscles, such as do not present the transverse striations... differ from the voluntary or striated muscles in their fibres being generally shorter than those of the latter; therefore fewer primary

cells arrange themselves together to form a secondary cell, and their fibres are commonly thinner and flat.

"I found in a human uterus, which contained a mature foetus, some long muscular fibres of the breadth of the common primitive fasciculi of voluntary muscles, which were so flat as scarcely to amount to 0.0010 to 0.0015 of a line in thickness".

Thus one of the earliest microscopical observations in the sequence we are tracing is associated through the person of Theodor Schwann with a much greater event, the enunciation of the cell theory of organic structure.

In 1840 a pupil of another great histologist, Purkyñe, Wilhelm Kasper by name, published at Breslau a doctoral dissertation, *"De structura fibrosa uteri non gravidi"*, in which he begins by stating that smooth muscle was known to exist in the pregnant uterus. Evidently either the observations of Lauth and Schwann were known at Breslau, or else Purkyñe had independently made the same discovery. This is more likely, because the pupil, Kasper, specially emphasizes the fact that he has found such cells in the non-pregnant uterus also, not mentioning that Lauth had already made the same statement. Kasper's brief description is however much more precise than that of Lauth. He says the fibers in the non-pregnant uterus are from 2 to 3 one-thousandths of a line in diameter, and resemble the muscle fibers of the intestine. The life history of these cells was now understood, for they were clearly seen to be present from the beginning, ready for the growth and function which pregnancy would impose upon them.

Incidentally , the mention in Kasper's dissertation of muscle fibers in the intestine and also in the arteries, shows that Purkyñe's school was almost as well informed on this subject as was Henle, to whom historians have given credit for the first mention (1841) of muscle cells in the blood vessels.

Eight years later (1848) Albrecht v. Kölliker published his great paper on smooth muscle, in which a full and correct description of the individual units was given for the first time and their distribution in the body fully explored. This work was based upon skillful isolation of such units from many tissues. This is the first really definitive statement of the histological nature of the uterine muscular tissue. The plates include good figures of muscle cells from the human uterus. In 1850 the young obstetrician F. M. Kilian, of Mainz, whose life was soon to be cut off by a surgical infection, rounded up the whole subject in an excellent paper on the structure of the uterus in animals. In the uteri of rabbits and guinea pigs, even in the first days of life, he found the characteristically spindle-shaped cells with long pointed ends and a central swelling occupied by the nucleus. In young animals Kilian found these cells so delicate and translucent that they could hardly be seen. In pregnant animals they grew to far greater size. Thus the point made by Kasper was amply confirmed, and the specific identity and the permanence of the uterine contractile elements were proved. A fundamental but beautifully simple lesson had been learned about the process of histogenesis, by which these

cells are differentiated in embryonic life, in anticipation of a long distant need.

This has been a story without a clear beginning and without a climax. Popular writers on science all too often depict research as a succession of exciting moments of illumination alternating with anxious effort. Experienced investigators will recognize here a more frequent course of events, in which there was less ecstasy than toil and the result nothing more exciting than a statement of facts about the structure of an organ. This result did not reveal itself in a flash of inspiration. It was built up by many men each of whom made only a small addition to knowledge, among them the great masters Schwann, Henle and Kölliker, the brilliant but short-lived Lauth and Kilian, the almost forgotten student Kasper. Working, each of them, only for the satisfaction of a problem solved, they did not know that upon their earnest toil would ultimately depend the favorable outcome of countless difficult labors, the lives of many mothers and their babes.

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LA SÉRIE ALLÉNOLIQUE DES OESTROGÈNES ARTIFICIELS

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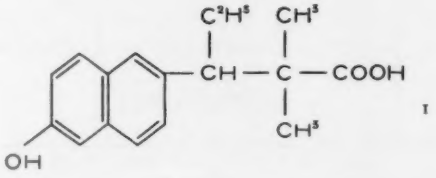
L'ENDOCRINOLOGIE sexuelle avance toujours d'un pas rapide. La technique des extraits glandulaires a conduit à la découverte des hormones naturelles pures. La connaissance de la constitution chimique de ces hormones a permis de les reproduire par synthèse en partant de composés chimiques plus simples. Nous vivons maintenant l'étape des hormones artificielles, et le travail est fort avancé en ce qui concerne les oestrogènes.

L'école anglaise a fourni le diéthylstilboestrol, l'hexoestrol, le diénoestrol; ensuite le triphényl-(chloro-ou bromo-)-éthylène. Les américains ont réalisé l'octofolline. Les Suisses ont ouvert le cycle D de l'équilénine de Girard pour aboutir à l'acide bisdéhydrodoisynolique (Miescher, 1944).

Une autre série d'oestrogènes artificiels a vu le jour, en 1947, dans mon laboratoire grâce aux efforts de mes collaborateurs chimistes A. Horeau et J. Jacques. C'est la série allénolique que nous avons ainsi dénommée pour honorer la mémoire du grand biologiste E. Allen.

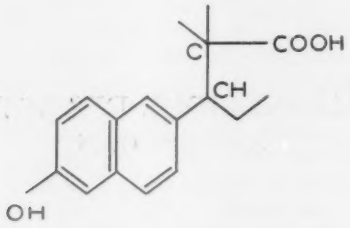
J'ai constaté que l'acide $\alpha\alpha$-diméthyl-β-éthylallénolique (formule 1), synthétisé par Horeau et Jacques, est très actif par la voie buccale chez le Rat castré à la dose de 1.5 à 2 gammas. Ce corps se prépare assez aisément à l'aide du β-naphtol, de l'acide propionique et de l'acide isobutyrique.



I

On peut l'écrire d'une autre façon (formule 2), et l'on retrouve alors la charpente des oestrogènes naturels, dont les cycles C et D son ouverts:

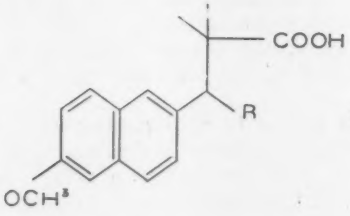
c'est une équilénine encore plus ouverte que l'acide bisdéhydrodoisynolique de Miescher.



(2)

Dans le cadre des recherches que nous poursuivons sur les relations entre la structure chimique et l'activité oestrogène, nous avons fait subir à la molécule de l'acide diméthyl-éthyl-allénolique certaines variations. Voici quelques résultats récemment obtenus grâce à l'habilité de Horeau et Jacques.

Considérons la molécule suivante:



(3)

si $R = C_2H_5$, il s'agit de l'éther méthylique de l'acide diméthyl-éthyl-allénolique.

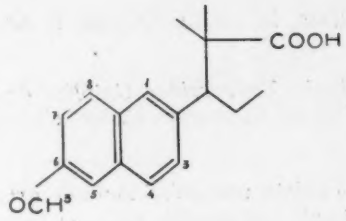
Il est possible de faire varier la longueur de la chaine R; voici les activités, sur le frottis vaginal du Rat, qui correspondent à ces variations:

R =	Dose en gammas	Résultats positifs
H	1000	0 %
CH^3	10	100 %
CH^3	5	0 %
C_2H^5	1,5	60 %
C_3H^7	1	70 %
C_3H^7	0,5	0 %
C_4H^9	20	20 %
C_4H^9	10	0 %

Nous pouvons constater que l'activité oestrogène augmente à mesure que la chaine s'allonge; mais cette activité passe par un maximum pour C_2H_5 et C_3H_7; et si l'on ajoute encore un carbone supplémentaire (C_4H_9), l'activité retombe.

Reprenons maintenant l'acide αα-diméthyl-β-éthyl-allénolique (formule 2), l'un des corps les plus actifs de la série.

Au cours de travaux difficiles, J. Jacques est arrivé à déplacer l'hydroxyle phénolique (ou son éther méthylique) dans toutes les positions possibles, en effectuant la synthèse de tous les isomères de l'éther méthylique de l'acide αα-diméthyl-β-éthyl-allénolique, isomères chez lesquels le méthoxyle prend, sur le noyau naphtalénique, toutes les positions réalisables, la chaine hydrocarbonée porteuse de la fonction acide restant en place. (voir formule 4).



(4)

Il ressort de cette étude que, de tous les corps synthétisés, l'acide αα-diméthyl-β-éthyl-allénolique est, de beaucoup, l'oestrogène le plus puissant; les plus actifs de ses isomères exigent des doses 200 fois supérieures pour provoquer une réaction vaginale identique chez la Rate castrée.

Position du méthoxyle sur la formule 4	Dose en gammas	Activité
en 6	1.5	5 cas positifs sur 8
en 7	500	0 » » » 5
en 5	250	4 » » » 4
en 5	125	0 » » » 4
en 4	500	0 » » » 5
en 1	500	5 » » » 6
en 1	250	0 » » » 4

Comment ne pas voir là une confirmation de l'importance de la forme de la molécule, et plus précisément de la ressemblance avec la structure spatiale des hormones naturelles? On sait en effet que, dans l'oestrone, l'oestradiol ou l'oestriol, l'hydroxyle phénolique occupe la même position que dans l'acide αα-diméthyl-β-éthyl-allénolique figuré en 2.

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MECANISMOS QUE INFLUYEN EN EL EFECTO DIURÉTICO DE LA RENINA

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PROBABLEMENTE no exista una substancia de origen animal, que pueda provocar un estímulo tan notable sobre el proceso excretor de orina, como la renina. Podría considerarse como un hecho singular el que esta propiedad la ofrezca una substancia destinada aparentemente a intervenir en la regulación de la presión arterial. Sin embargo, es sabido que otras substancias hipertensoras como la adrenalina y la tiramina, son capaces de inducir un aumento muy apreciable del flujo urinario (Hughes-Jones, 1949); en cambio otras, como la vasopresina (Motzfeldt, 1917), la enteramina (Erspamer, 1952) provocan una reducción muy efectiva de la diuresis. Los hechos experimentales indican que los mecanismos de acción de estas substancias, son distintos y dependen de las condiciones experimentales y de las dosis que se emplean. A este respecto, es llamativo el caso de que la vasopresina, la hormona antidiurética por excelencia, que cuando es inyectada en ratas normales con libre acceso al agua, y en dosis elevadas (del orden de 0.2 U), determina una diuresis 2 a 4 veces más alta que la normal. Este efecto diurético que se observa cuando las dosis administradas son exageradas, parece depender de la propiedad hipertensora de la misma (Ham y Landis, 1952; Croxatto y col., 1953).

Pickering y Prinzmetal (1940), han demostrado que la diuresis producida por la renina no es una simple consecuencia del aumento de la presión intraglomerular. Hughes-Jones afirma además, que la hipertensina (por vía intravenosa, en conejos), tendría un efecto sobre la diuresis idéntica al de la renina y como esta última, produciría una inhibición de la reabsorción del agua, cloro y sodio en el tubulo renal. Puede considerarse como un hecho suficientemente demostrado que también en la rata, la renina ejerce sobre estos iones y el agua un efecto similar (Sellers y col., 1951).

No es extraño así, que en animales que reciben en la bebida dosis elevadas de ClNa, la renina incrementa considerablemente el efecto diurético.

A pesar de que la suposición de Hughes-Jones, aparece muy bien apoyada por los hechos experimentales en el conejo, en la rata no está

suficientemente probado que los efectos de la renina sobre la diuresis se expliquen por la intervención de la hipertensina. En efecto, en la rata, la inyección de hipertensina por vía intraperitoneal, no produce variación significativa sobre la diuresis. Por vía subcutánea y en dosis repetidas, puede revelar un efecto que recuerda al obtenido con dosis moderadas de renina (Valdés, 1953).

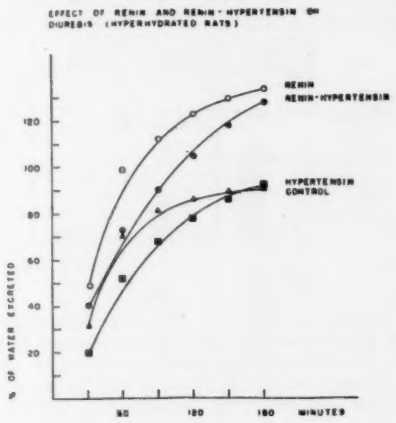

FIG. 1. — *Cada curva representa el tanto por ciento de excreción urinaria de 4 ratas (test Burn). La dosis de renina fué de 1 ml (25 U) por vía intraperitoneal y por rata. La dosis de hipertensina administrada fué de 0.1 ml (5 U) por vía subcutánea. El grupo control se inyectó con 1 ml de sol. ClNa 0.9 %. Las inyecciones fueron hechas dos horas antes de hidratar los animales.*

Además, hemos podido destacar el hecho que ratas sometidas a una sobrecarga acuosa e inyectadas con renina (test de Burn, 1931), responden de un modo complejo frente a una inyección única. Si la renina se introduce por vía intraperitoneal, simultáneamente con la administración de agua, se provoca un retardo acentuado de la excreción de orina. Un hecho diametralmente opuesto sobreviene si la inyección de renina precede por 2 a 4 horas a la hiperhidratación (Croxatto y col., 1952).

El efecto inmediato de la renina inyectada intraperitonealmente que se traduce en una frenación de la eliminación de orina, aparece ligado a la formación de hipertensina. Esta se liberaría en los primeros momentos, en el período relativamente pasajero en que la presión arterial se eleva. En una rata normal, el aumento de la presión arterial, que sigue a la inyección intraperitoneal de renina es fugaz, si se compara con el período de 4 a 10 horas durante el cual es posible comprobar un marcado estímulo de la diuresis.

La hipertensina (5 U) inyectada conjuntamente con la renina (25 U) o poco después, disminuye el efecto diurético que esta última tiene en una rata hiperhidratada (Fig. 1). Este resultado favorece el punto de

vista de que la fase antidiurética en los primeros instantes se debería al efecto vasoconstrictor de la hipertensina. Hughes-Jones ha insistido que ambas substancias en el conejo (por vía intravenosa) inducen un efecto antidiurético inicial y pasajero. Pasada esta fase, sobreviene la descarga poliúrica. No se sabe si este efecto inhibidor de la reabsorción

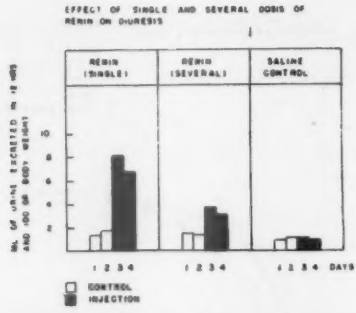

FIG. 2. — *Las barras blancas representan la excreción de orina en los días en que los animales no son inyectados; las negras representan la diuresis en los días en que son inyectados. El primer grupo fué inyectado con una dosis única de 25 U de renina (1 ml). El segundo con 5 dosis de 12.5 U de renina (0.5 ml) cada dos horas. El tercer grupo fué inyectado con una sol. ClNa 0.9 %.*

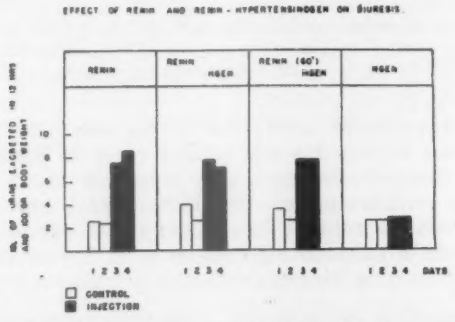

FIG. 3. — *Idem Fig. 2. El primer grupo fué inyectado con 25 U (1 ml) de renina por vía intraperitoneal. El segundo con 25 U (1 ml) de renina más 1 ml de una solución concentrada de hipertensinogeno, simultáneamente. El tercero con 25 U (1 ml) de renina y 60 minutos después con 1 ml de hipertensinogeno. El cuarto con 1 ml de hipertensinogeno.*

tubular del agua se debe a un producto derivado de la hipertensina; o a que en el riñón perduran los cambios vasculares por un tiempo mayor que lo que dura la hipertensión arterial y son los fenómenos vasculares intrarrenales los que perturban la reabsorción del agua. En todo caso es un hecho suficientemente esclarecido que el máximum de efecto diu-

rético de la renina se alcanza durante una fase en que la presión arterial se ha restablecido totalmente y que la acción antidiurética coincide con la etapa hipertensiva.

La sucesión de una fase antidiurética y de una diurética inducidas por la renina, explica el resultado curioso que el efecto diurético de una inyección de renina puede ser anulado con una segunda dosis a condición de introducir esta última en el momento en que la primera debe iniciar la fase diurética. Una dosis única de renina origina un efecto estimulador

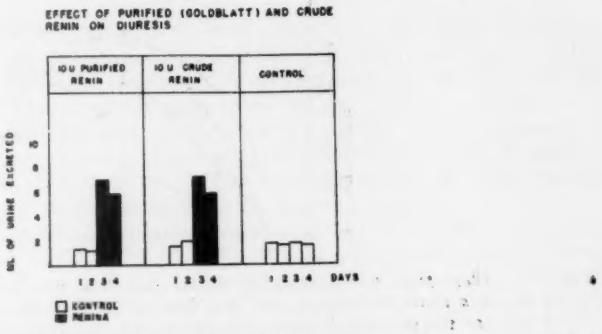

FIG. 4. — *Idem Fig. 2. El primer grupo es inyectado con 10 U de renina purificada (242 Goldblatt). El segundo grupo fué inyectado con igual unidades de renina cruda. El tercer grupo sirvió de control.*

más intenso de la diuresis que la de varias dosis distribuídas en 2 ó 4 inyecciones sucesivas, siempre que sean aplicadas a intervalos de tal modo calculados que, cuando se inicia el efecto estimulante de la inyección precedente, se administre la dosis siguiente y así sucesivamente (Fig. 2).

El efecto estimulador de la diuresis no se afecta con la inyección previa de hipertensinógeno (intraperitoneal) en dosis relativamente considerables, lo que por cierto contradice que el efecto de la renina esté condicionado a la cantidad de hipertensina que pueda formarse con cada dosis de renina inyectada (Fig. 3).

Si todavía no sabemos a ciencia cierta que en la rata sea la hipertensina la que en último término explique las fases antidiurética y diurética de la renina, está también pendiente el experimento que permita conocer el efecto que tiene sobre la función excretora del riñón la inyección continuada de hipertensina, que reproduzca la liberación gradual de la substancia vasoconstrictora cuando la renina inyectada actúa sobre el hipertensinógeno circulante.

Hasta ahora no se ha obtenido la renina al estado de completa pureza como para poder descartar que la acción sobre la función excretora del riñón no se debe a una substancia extraña. Sin embargo, los ensayos hasta aquí practicados, dejan entrever que no es una impureza de la renina la que produce los fenómenos sobre la diuresis, y que es, al parecer, la mis-

ma substancia que influye sobre la presión arterial. En efecto, si se compara en dos preparados de renina, en diversos estados de purificación, el efecto vasoconstrictor y el diurético, se encuentra que la relación entre ambos se mantiene. Esta conclusión es válida al menos para la renina de una misma especie animal. En el gráfico Nº 4 se compara el efecto sobre

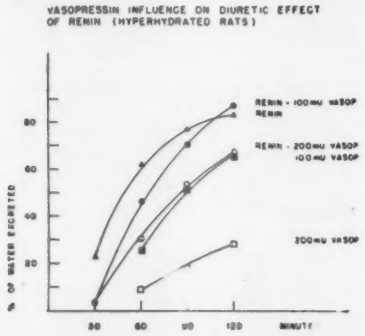

FIG. 5. — *Idem Fig. 1. La dosis de renina fué de 1 ml (25 U) por vía intraperitoneal. Las dosis de vasopresina son las que se indican en el gráfico y se inyectaron por vía intraperitoneal. La renina fué inyectada dos horas antes y la vasopresina una hora antes de hidratación.*

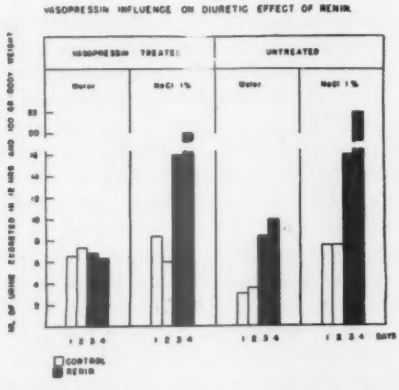

FIG. 6. — *Idem Fig. 2. El primer grupo recibía agua pura en la bebida. El segundo ClNa 1% en la bebida. Estos dos grupos fueron tratados durante 10 días con 0.2 U de vasopresina por vía subcutánea. El tercer y cuarto grupo recibieron agua y ClNa 1 % respectivamente, pero no fueron inyectados con vasopresina. Las barras negras indican la diuresis correspondiente a los días en que los animales son inyectados con 25 U (1 ml) de renina. Durante estos días se suspende el tratamiento de vasopresina, de los grupos primero y segundo.*

la diuresis de una renina obtenida por la técnica de Braun-Menéndez y col. (1943), con la purificada y proporcionada por Goldblatt *, en dosis que producían efectos equivalentes sobre la presión.

Frente a la acción diurética, natriurética y clorurética de la renina era de importancia establecer qué respuesta se obtenía con la administración de vasopresina, que aumenta la reabsorción tubular del agua pero no la del sodio y el cloro. Se puede poner de manifiesto un antagonismo, y como era de esperar, las inyecciones en ratas hiperhidratadas, de renina y vasopresina en diversas relaciones de dosis y de tiempo, producen efectos que corresponden a una neutralización cuantitativa de ambos principios (Fig. 5).

Aun cuando no es el propósito de este trabajo el adelantar todos los detalles técnicos, se puede consignar, que en animales que tienen libre acceso al agua (normalmente hidratados) la inyección diaria de 0.2 U de vasopresina, por el espacio de 10 días produce una franca resistencia a la renina, la que no manifiesta el efecto diurético típico. Esta incapacidad de los animales para revelar esta acción, parece depender de un empobrecimiento acentuado de cloro o de sodio que las inyecciones de vasopresina determinan después de un largo período de administración.

Si las ratas son forzadas a una ingestión de ClNa agregado en el agua de bebida (1 %) y se someten al tratamiento de vasopresina en las mismas dosis y por el mismo tiempo, se observa que la renina da lugar al efecto diurético habitual, de una intensidad semejante a la que se alcanza en el animal que no se inyecta con vasopresina (Fig. 6).

La extirpación de la hipófisis en ratas, que induce el estado de diabetes insípida, por exclusión de la vasopresina, no hace a los animales más sensibles a la renina. El animal hipofisectomizado sometido a la hiperhidratación se comporta como el surrenalectomizado, revelando una grave incapacidad para excretar el agua en exceso. La administración de renina, lejos de favorecer la eliminación del líquido, agrava el estado del animal hipofisectomizado. La cortisona es capaz de corregir en parte este déficit de excreción, pero el restablecimiento de la poliuria renínica a un nivel cercano al normal, se obtiene con la administración de ClNa al 1 % en la bebida.

La extirpación de las suprarrenales abole totalmente la capacidad de los animales para responder a la renina, tanto en normales como en hiperhidratados. La cortisona y la desoxicorticosterona restauran la capacidad alcanzando la excreción del agua una velocidad cercana a la observada en los normales.

Ni la extirpación de la tiroides, ni el hipertiroidismo parecen afectar la poliuria renínica (Sandoval, 1953).

Los hechos experimentales dejan en claro que las condiciones que más influyen sobre la poliuria renínica son: el nivel de ingestión de ClNa y la mayor o menor disponibilidad de hormonas corticosuprarrenales.

Cuando se asocia un exceso de desoxicorticosterona y de ClNa a un estado de hipertensión arterial (nefrectomía unilateral y envoltura del

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* Agradecemos al Dr. H. Goldblatt la gentileza de proporcionarnos el material purificado.

otro riñón con celofán), con reducción efectiva del parenquima renal, el efecto diurético de la renina alcanza un grado extraordinario, observándose que el animal excreta en las 24 horas siguientes un volumen de orina cercano al 50 % de su propio peso. Es digno de atención además, el hecho que la administración prolongada de renina por vía intraperitoneal, aparece en nuestros experimentos, muy bien tolerada sin agravar la hipertensión y sin grandes cambios de peso, en contradicción con los efectos tóxicos descriptos por Masson y col. (1950), con diversos extractos renales que contenían renina.

La investigación, tanto clínica como experimental, todavía no ha resuelto el enigma que encierra el mecanismo etiopatogenético de la hipertensión esencial. No se conoce en definitiva el papel de la renina como factor desencadenante o mantenedor del estado hipertensivo. Otros factores tienden a ser considerados como de gran importancia como son los que alteran el metabolismo del sodio, del cloro y del agua. Entre los factores que influyen en el desarrollo de la hipertensión, se destacan las hormonas corticosuprarrenales (Selye, 1946); la existencia de una substancia antidiurética cuya excreción está aumentada en los pacientes hipertensos y en animales con hipertensión nefrógena (Ellis y Grollman, 1949) y un aumento del espacio extracelular en los hipertensos (Grollman y col., 1953). Todos estos hallazgos muestran una íntima conexión entre la hipertensión y el metabolismo del agua y las sales. La interferencia de la renina en la función excretora renal del agua y de los elementos salinos nombrados, lejos de disminuir la importancia de la renina, pueden justificar aún más, el que se continúe investigando hasta qué punto el esquema Goldblatt-Houssay puede explicar los hechos más sobresalientes del cuadro hipertensivo.

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RELAÇÕES GENÉTICAS ENTRE AS GÓNADAS E A GLÂNDULA CORTICO-SUPRARENAL

A. CELESTINO DA COSTA

(*Lisboa*)

É PONTO incontroverso que entre as gónadas e o cortex suprarenal existem correlações funcionais. A existência dessas correlações fundamenta-se em observações de ordem fisiológica e patológica, bem como no parentesco bioquímico das chamadas "hormonas" sexuais e corticais. Não é porém desses argumentos bioquímicos, experimentais e clínicos que nos vamos ocupar neste trabalho, mas sim de outros tirados da morfologia.

Começaremos por recordar que a parecença histológica entre o corpo amarelo e a suprarenal sugeriu a Prenant e a Born, ainda nos fins do século passado, a ideia de atribuir àquele orgão do ovário uma função endocrínica, ideia que se mostrou ser exacta e fecunda. Mas a citologia dos elementos intersticiais do testículo e do ovário, pela sua semelhança com a das células cortico-suprarenais logo veio trazer bons argumentos para os que procuravam, como Bouin e Ancel, localizar nesses elementos a sede da secreção interna daqueles orgãos.

Por seu turno, a embriologia revelara, há muito, que os esboços cortico-suprarenais e gonádicos são vizinhos no epitélio do celoma de que uns e outros derivam, a ponto de nós próprio e Goormaghtigh termos tido, em certos casos, dificuldade em bem delimitar um de outro esboço.

Não satisfeitos com a demostração desta íntima vizinhança, certos autores têm ido mais longe na compreensão do parentesco embriológico das gónadas e do esboço cortico-suprarenal.

Para melhor compreensão do estado actual desta interessante questão do parentesco embriológico dos orgãos sexuais e da suprarenal, recordarei que ovário e testículo se diferenciam no esboço gonádico que faz saliência para a cavidade do celoma, o primeiro à custa da porção cortical da gónada ou cortex, o segundo à custa da porção central ou medula (Witschi). Segundo a teoria clássica, cortex e medula seriam produtos da proliferação dos cordões celulares formados, para dentro do mesenquina, pela proliferação das células do epitélio celómico da área, ou eminência genital. No caso do testículo, a porção desses cordões

mais afastada do epitélio celómico iria dar o conjunto dos tubos seminíparos, oriundos, pois, unicamente, na medula do esboço, visto que à periferia a formação precoce da futura albuginea, de natureza mesenquimatosa, impediria qualquer diferenciação à custa do cortex gonádico. No caso, porém, do ovário, um primeiro surto proliferativo daria uma medula rudimentar, cujos restos no adulto seriam os tubos medulares descritos pelos histologistas ou mesmo, na Toupeira, como o mostrou Tourneux, um rudimentaríssimo testículo, não funcional. Novo, ou novos surtos proliferativos dos cordões celómicos dariam os cordões de Valentin-Pflüger e os folículos primordiais de que se formam depois os folículos ováricos definitivos. Tal é a teoria clásica segundo a qual também o esboço cortico-suprarenal, situado medialmente em relação ao gonádico, embora muito próximo, se separa precocemente do epitélio, e vai relacionar-se, uma vez independente, com os elementos paraganglionais que, nos Mamíferos, darão a medular suprarenal.

Entre as opiniões divergentes da clássica, citaremos primeiramente a de Fischel, segundo a qual os cordões sexuais proviriam do mesênquima subjacente ao epitélio do celoma e não dêste. Embora a noção de "cordões sexuais" seja um pouco esquemática, pois a proliferação em que se delimitarão as unidades histológicas da gónada assume um feitio bastante compacto onde difícilmente se destrinçam os tais cordões, a natureza únicamente mesenquimatosa dessa proliferação é contestável e as observações concordam, de preferência, com a natureza mesotelial. Aliás, também se têm atribuido ao mesênquima a formação directa dos cordões cortico-suprarenais. Num trabalho nosso, assaz recente, fizemos notar que o esboço cortico-suprarenal é nìtidamente basófilo nas primeiras fases, como o são o epitélio do celoma, quer geral, quer genital, e os tubos mesonefríticos, ao passo que a basofilia é pouco acentuada no mesênquima.

Mais directamente relacionada com o assunto deste trabalho é a opinião de Witschi que só para o ovário admite origem no epitélio celómico. Tanto o testículo como os elementos tecais do ovário e o esboço cortico-suprarenal teriam sua origem num blastema mais profundo subepitelial. Dantschakoff via, por seu lado, a origem da medula gonádica no mesonefros.

Recentemente, Vannini, baseando-se em observações feitas em Batráquios, Aves e Mamíferos (estas de colaboração com Cessi) sustenta não só que a medula gonádica (isto é, o testículo) não provém do mesonefros (o que, em relação ao cortex suprarenal, já há muito estava posto de parte), fundamentando em parte essa opinião em estudos experimentais (excisão precoce do rim primitivo em gerinos da Rã, o que não impede a formação do testículo) como também descreveu a formação do cortex suprarenal a partir do epitélio do celoma, no embrião do Frango, após se ter constituido o mesonefros. Ora nesse esboço celómico diferenciar-se-iam precocemente suas duas partes dorsal e ventral, aquela dando pròpriamente a parte cortical da suprarenal, esta produzindo a medula gonádica. Nos Mamíferos, porém, o cortex suprarenal teria um duplo modo de formação, visto que as porções mais caudais do respectivo esboço, em vez de provirem directamente do epitélio celómico se formariam à

custa do esboço da medula gonádica, já prèviamente constituido. Como Witschi, pois, embora com fundamentos diferentes, Vannini encontra origem comum no esboço cortico-suprarenal e no testículo, o que lhe dá bases morfológicas para a compreensão de certos parentescos bioquímicos e fisiológicos entre a hormona cortical (desoxicorticosterona) e a testicular. Escusado será insistir nas deduções que se podem tirar a respeito da função androgénica do cortex que tantos admitem como fisiológica.

Outra opinião no mesmo sentido é a de Velican, que admite, no embrião humano, dois surtos diferentes dos esboços cortico-suprarenais. Parte desses esboços, de constituição metamérica e de orígem celómica, daria o cortex suprarenal pròpriamente dito; a outra, difusa, mas também de origem celómica, vizinha dos esboços genitais, daria elementos mesenquimatosos de que proviriam também as células intersticiais do ovário e do testículo.

A atribuição de dois surtos diferentes na formação do esboço cortico-suprarenal foi há tempos defendida por Uotila que atribuia ao cortex fetal humano uma origem mais precoce e diferente da do cortex definitivo. Mas Gruenwald, que foi verificar esta teoria, também em material humano infirmou-a.

Nós não dispusemos desse material. O nosso material provém de séries completas de embriões de Cobaia, a que se devem juntar outros embriões de Ouriço, Rato, Gato e Morcegos vários. Verificamos, com efeito, que o esboço cortico-suprarenal se constitui a partir de proliferações diversas, em região extensa, do epitélio do celoma, entre a raiz do mesentério e o esboço da gónada.

Essas proliferações, que aparecem na Cobaia no 20º dia, separam-se do peritoneo, concentram-se à roda do sistema de vénulas subcardinais, formam um conjunto de cordões epiteliais nìtidamente basófilos, como atraz dissemos, que é logo abordado e penetrado pelos cordões paraganglionares que vêm do esboço proto-simpático juxta-aórtico.

Quanto ao esboço gonádico, nesse 20º dia de vida embrionária da Cobaia, constitui uma longa fita de mesotélio celómico espessado, também basófilo como os outros derivados celómicos, situada do lado medial do corpo de Wolff e vizinha do esboço cortical. Mas essas relações de vizinhança atenuam-se ràpidamente com interposição de mesênquima entre os dois esboços, principalmente desde que, aos 21 e 22 dias, a fita germinal se torna saliente, constituindo o que, em cortes transversais, se pode designar como eminência germinativa.

Não mais se vêem conexões entre os esboços gonadico e cortico-suprarenal. Este pode ficar durante algum tempo em ligação por um ou outro pedículo com o epitélio do celoma, mas en região separada daquela em que está o esboço da gónada, o qual se relaciona aqui e ali com o corpo de Wolff, constituindo essas ligações as conexões urogenitais.

Nenhuma das nossas observações nos permite pois admitir que a porção medular da gónada provénha de esboço comum ao do cortex. Esses esboços são vizinhos ao princípio, mas ràpidamente se separam. A vizinhança de origem ajuda-nos a compreender o parentesco fisiológico dos orgãos, mas nada mais consentem.

Subsiste, porém, um outro ponto. Vem a ser que a parte dos orgãos

sexuais a que mais provàvelmente se atribui a secreção interna masculinizante ou feminizante é constituida pelas células intersticiais testiculares ou ováricas que geralmente —mas não unânimemente— são consideradas como conjuntivas, mesenquimatosas. A procura pois dum parentesco embriológico como base para a compreensão do parentesco hormonal, bioquímico, deberá fazer-se entre essa origem mesenquimatosa dos elementos androgénios e estrogénios e dos elementos mesoteliais celómicos do cortex suprarenal.

Esta é uma questão que necessita de novas e delicadas investigações, mas, a nosso ver, diferente da maneira como até aqui se tem querido considerar esse parentesco.

EXTENDIDOS DEL SEDIMENTO URINARIO *

Su utilidad para determinar la actividad estrogénica

E. B. DEL CASTILLO Y J. ARGONZ

En el año 1946, del Castillo, Argonz y Galli Mainini establecieron el estrecho paralelismo existente entre los extendidos del sedimento urinario y los extendidos vaginales, siendo los mismos realizados en pacientes en las distintas etapas de su desarrollo genital y en diferentes casos con insuficiencia estrogénica. En otra publicación, en 1948, fueron completadas estas observaciones con los resultados obtenidos en una amplia utilización del método, para lo cual se creó una técnica especial. Prosiguiendo sus estudios los mismos autores en 1949 pudieron determinar el origen de las células pavimentosas del sedimento urinario, comprobando que eran debidas a la descamación del epitelio de la mucosa uretral en sus 2/3 anteriores.

Con anterioridad a estas publicaciones otros autores, Biot y Beltrán en 1944 y Papanicolau en 1945, habían estudiado, los primeros, el número de células del sedimento urinario en la ovulación y el segundo sus caracteres morfológicos en las neoplasias de las vías urinarias, sin mencionar ambos el paralelismo de los elementos citológicos vaginales y uretrales y su empleo como método diagnóstico en las perturbaciones menstruales.

Con posterioridad a nuestras publicaciones han aparecido varios trabajos relacionados con la citología del sedimento urinario, pudiendo mencionar entre ellos el del Dr. Wasserman, que corrobora nuestras observaciones, el del Dr. Papanicolau en 1948, quien aplica el estudio del sedimento urinario para el diagnóstico del embarazo y señala las ventajas de este método sobre las que ofrecen los extendidos vaginales para este propósito; en 1950 Mac Callin y Taylor estudian los cambios citológicos del sedimento urinario durante el ciclo menstrual y el embarazo; en 1951 Alvan Foraker estudia el efecto del estilbestrol sobre las células del sedimento urinario; en 1952 Taylor y Mc. Callin establecen el paralelismo existente entre la citología del sedimento urinario y la histología del endometrio; en 1953 Rosen publica sus observaciones sobre la correlación entre la citología de la vagina, del sedimento urinario y de la mucosa bucal en niños recién nacidos y Talbot y colaboradores, en su reciente tratado so-

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bre endocrinología infantil, incluyen el estudio de los extendidos urinarios entre los métodos de examen para determinar la actividad estrogénica en la niña. Finalmente, en el año 1953, Lencioni aplica el método estadístico para comprobar si existe el paralelismo que establecimos en nuestras publicaciones entre los extendidos vaginales y los del sedimento urinario y llega a la conclusión, después de estudiar a 68 mujeres, que el método del sedimento urinario proporciona informaciones valederas en ginecología y endocrinología y tiene un estrecho paralelismo con el método de los extendidos vaginales, afirmación fundada en comprobaciones estadísticas.

Material y método

Considerando que el método ha entrado en la práctica de rutina de varios servicios dedicados al estudio de la endocrinología ginecológica, el material estudiado es muy amplio y comprende a mujeres normales desde la temprana niñez hasta la menopausia avanzada, a pacientes que sufren trastornos del ciclo menstrual por múltiples causas y a pacientes que desean resolver el problema de su esterilidad.

El método está basado esencialmente en la utilización de la orina de la primera micción matinal, aunque no existen inconvenientes en utilizar cualquier otra micción, siempre que hayan transcurrido algunas horas para conseguir mayor riqueza celular. Esta orina puede ser centrifugada o filtrada, utilizando papel de filtro o su pasaje por algodón, extendiendo el material conseguido sobre un porta-objetos que se fija de inmediato en licor de Hoffman durante 5 minutos; luego se cubre con el colorante de Shorr durante 2 minutos, se seca y puede hacerse la lectura del extendido. Esta técnica simple y rápida, que sólo necesita pocos minutos para su ejecución, permite aplicarla en la práctica diaria con los pocos elementos de laboratorio que hemos mencionado.

Diferentes autores han propuesto modificaciones utilizando otros métodos de fijación y distintos colorantes que no creemos ofrecen mayores ventajas. No obstante ello, es indudable que la observación de Lencioni de que es posible utilizar extendidos, secados al aire ambiente, con el simple pasaje previo por alcoholes acidulados antes de colorearlos, es digna de tenerse en cuenta porque facilita la aplicación y difusión del método.

Cuando interesa conservar los preparados, se procede de acuerdo a la técnica conocida de pasajes de alcoholes de mayor concentración y de xilol para montar con bálsamo de Canadá.

Las críticas que se hicieron originariamente a nuestro método por la forma de recoger el material que se consideraba podía estar contaminado con las secreciones vaginales, han perdido todo valor después de nuestras investigaciones sobre la mucosa uretral que nos permitieron conseguir directamente de este conducto el material y comprobar su similitud con el que se conseguía por la micción expontánea.

Aplicaciones del método

El estudio del sedimento urinario permite apreciar esencialmente la actividad estrogénica en base a las modificaciones citológicas bien conocidas desde los estudios de Papanicolau y Shorr sobre los extendidos vagi-

nales. La hemos utilizado en niñas antes de la edad de la pubertad, en cuyo caso se caracteriza por su pobreza celular y la sola presencia de células intermedias y profundas con núcleo grande, en esta etapa de la vida permite predecir la aparición de la menarquia, ya que muchos meses antes empiezan los extendidos a sufrir modificaciones apareciendo mayor cantidad de elementos celulares y de tipo más evolucionado. Es de suma utilidad en el estudio de los interxesos, habiendo encontrado en muchos casos de hipospadias sedimentos de tipo estrogénico, lo que permitió orientar mejor las investigaciones ulteriores. Es sabido que la uretra masculina no presenta descamación valorable para fines diagnósticos, salvo cuando el paciente es tratado con dosis adecuadas de estrógenos, en cuyo caso se produce una eliminación de células de tipo superficial.

En las mujeres normales es posible seguir ciclos completos, con el estudio diario de los extendidos urinarios, como lo hemos realizado muchas veces, y se consigue observar las mismas modificaciones que se han descripto en los extendidos vaginales, inclusive establecer con cierta precisión la fecha de la ovulación en la cual las células tienen bordes netos, se colorean mejor, abundan las superficiales y no se aglomeran en acúmulos. Por estas circunstancias el método puede emplearse en el estudio de la esterilidad, para determinar si estamos en presencia de un ciclo anovulador.

En la menopausia es también de utilidad, ya que los extendidos tienen características especiales: predominio de células intermedias, presencia de mucus y leucocitos y grandes variaciones en el número y las características de las células superficiales.

Pero indudablemente donde el método adquiere mayor importancia es en el estudio de los trastornos menstruales, ya que permite establecer con bastante precisión el grado de actividad estrogénica y orientar el diagnóstico. Recordemos los extendidos atróficos de las amenorreas primarias, del sindrome del ovario rudimentario y de las insuficiencias ováricas primitivas o secundarias y la riqueza estrogénica de las amenorreas de causa uterina, con lo cual evitaremos errores de diagnóstico y tratamiento. Mencionaremos los extendidos tróficos de la metropatía hemorrágica y de los ovarios poliquísticos; el tipo de extendido en la fibromatosis uterina y en el embarazo, tan diferentes ya que en el primer caso presenta un grado excelente de actividad estrogénica con abundantes células cornificadas y en el segundo predominan las células intermedias con la presencia de células "naviculares", con lo cual y merced al extendido podremos dilucidar la duda bastante frecuente que se presenta cuando debemos afirmar si estamos en presencia de un fibroma o de un embarazo.

Otra aplicación de orden práctico se obtiene cuando se estudia la evolución de los extendidos bajo la influencia de la terapéutica estrogénica, ya que podemos apreciar la actividad del medicamento y el grado de reacción que se produce en la paciente.

Resultados y conclusiones

Para determinar la utilidad de este método mencionaremos las palabras textuales de Papanicolau en su trabajo sobre diagnóstico del embarazo. Dice este autor: "me ha impresionado la gran uniformidad de los

extendidos del sedimento urinario comparados con los extendidos vaginales", y, más adelante, agrega: "la aparición de cambios significativos en la citología de los extendidos puede ser más fácilmente apreciada en el sedimento urinario que en el extendido vaginal" y "el sedimento urinario prueba ser así más eficaz para ensayar la actividad de los estrógenos que el extendido vaginal". Con esto debía bastar, dada la indudable autoridad del autor, pero insistiremos sobre las ventajas y desventajas de este método como resultado de la vasta experiencia adquirida en más de siete años de su empleo.

Las ventajas son su realización simple, sin molestias para la paciente, hecho digno de tenerse en cuenta en las niñas, siempre reacias en nuestro medio al examen genital; la mayor limpieza de los extendidos que permite más fácilmente el recuento y estudio de la morfología celular y la uniformidad de los cuadros citológicos que no son modificados por lavajes, coito, procesos inflamatorios o parasitarios como ocurre en los extendidos vaginales. Las desventajas que pueden imputársele pertenecen al estudio de algunas pacientes que tienen muy poca actividad estrogénica en las que muchas veces el número de células es muy escaso y son más difíciles de encontrar las células espinosas profundas. Consideramos que este problema podrá ser resuelto mejorando la técnica para conseguir sedimentos más ricos, evitando el arrastre celular en el proceso de fijación y coloración.

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RELATION OF STILBOESTROL TO NATURALLY OCCURRING SUBSTANCES

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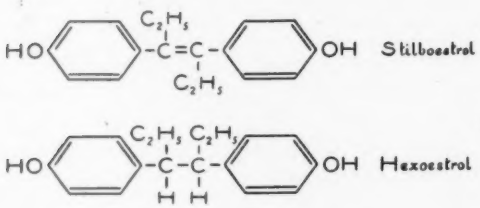
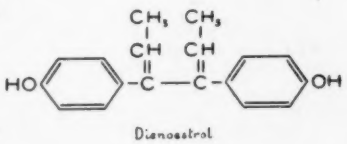
THE EXPERIMENTAL work leading to the synthesis of stilboestrol has been published on a number of occasions (Dodds) and it is only necessary to make the briefest reference to this subject in the present communication.

It was the fact that five oestrogenic substances were known to occur in nature that led the first workers (Cook, Dodds and Hewett) to experiment with the simple phenanthrene compounds. This led to the discovery of the first synthetic oestrogenic substance, namely 1-keto-2:3:4 tetrahydrophenanthrene. The next step (Dodds and Lawson) consisted in showing that the phenanthrene ring system was not necessary for oestrogenic activity and finally an investigation of the activity of substituted stilbene derivatives was undertaken on purely chemical grounds.

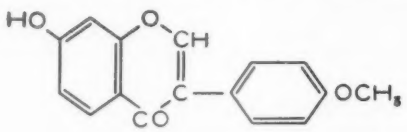
As a result of the investigations three very potent synthetic oestrogens were produced (Dodds, Golberg, Lawson and Robinson). Stilboestrol, dienoestrol and hexoestrol have the following formulae and have found extensive use in both clinical and experimental work.





Up to the present time there has been no explanation forthcoming for the activity of this groups of substances. Stilbene itself does not occur in any known natural product and, therefore, substituted stilbene such as stilboestrol bears no resemblance to any known naturally occurring compound.

Recently the position has been altered by the work on subterranean clover. In Australia (Bennetts & Underwood) it has been known for some considerable time that certain pastures led to a diminution in the fertility of sheep and the defect has been shown to be in the ewes, the potency of the rams remaining quite normal. Histological examination of the uterine mucosa showed a condition of hyperplasia very similar to that occurring under the influence of heavy oestrogenic administration. It was also known that these pastures were overgrown with subterranean clover, a very rich form of feeding stuff and it was only natural that an investigation should be made into the possibility of this constituent in the pasture supplying the oestrogens. Extracts were made of subterranean clover and it was very clearly shown that an oestrogen could be extracted from this material. This substance was capable of producing oestrus when injected into ovariectomised rats or mice in exactly the same way as the naturally occurring oestrogens. Extensive work has been conducted on the active constituent of subterranean clover. There is considerable evidence (Bradbury & White) that at least one oestrogenic substance is the flavone genistein. This has the following formula:



It can be seen that it is closely related to stilbene in structure; if the oxygen bridge be opened then a stilbene compound is obtained. Various derivatives of genistein have been found and in woods similar types of compounds are encountered in considerable quantity.

These interesting observations from Australia stimulate reflections that there may be possible precursors of stilboestrol like substances in nature.

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NEUROMUSCULAR ALTERATIONS PRODUCED BY MOTOR-NERVE SECTION

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THE FUNCTIONAL disturbances produced by section of a nerve axon may be grouped as follows: (1) Modifications of the central stump; (2) Alterations of the peripheral stump and; (3) Alterations of the structures innervated by the severed axon.

Although the phenomena under discussion have a general interest we shall limit our discussion to the neuromuscular system. A thorough bibliographical review is beyond the scope of this paper. Actually we shall present only some physiological problems that are being stated with regard to the degeneration of nerves.

RETROGRADE DEGENERATION

The disturbances produced in the central stump, which have been called retrograde degeneration, have been partly studied from a functional point of view. Acheson, Lee and Morison (1942) found a decrease in the amplitude of the phrenic nerve discharges during this degeneration. These alterations progressed up to 2 or 3 weeks after the nerve section to recover afterwards in such a manner that responses were normal about 2 months after the section of the nerve. Campbell, Mark and Gasteiger (1949) hace demonstrated that this type of degeneration modifies reflex responses and that anti-dromic responses presented low voltage potentials.

WALLERIAN DEGENERATION

Alterations produced in the distal stump have been labeled Wallerian degeneration as an homage to A. Waller who in 1852 performed some studies about structures undergoing degeneration.

From a physiological point of view, three types of alterations during Wallerian degeneration could be analized: (a) Disturbances in the conduction of nerve impulses; (b) biochemical changes of nerves, and (c) alterations of synaptic function.

(a) The characteristic of nerve impulses during the course of Wallerian degeneration have been studied by several investigators (Rosenblueth and Dempsey, 1939; Erlanger and Schoepfle, 1946; Gutmann and Holubár, 1950). All these authors agree that the conduction of nerve impulses persists up to 70 or 80 hours after nerve section, that conduction velocity and excitability remain almost normal and that the height of the spike potential slowly decrease in height until it stops conducting almost abruptely. On the other hand they disagree with regard to the temporal course of degeneration. Thus, Rosenblueth and Dempsey (1939) infer from their results that disturbances follow a centrifugal course. Erlanger and Schoepfle (1946) insist that the alterations may appear simultaneously in all loci of the degenerating nerve, but these alterations would be more frequent toward the periphery. Gutmann and Holubár, (1950), suggest that any given locus of the axon may stop conducting suddenly. Considering that the inferences reached by these authors seem to be licit, it is difficult, for the moment, to explain the different conclusions. Further studies would probably elucidate this problem.

(b) The biochemical changes during this process have been studied by several authors. Most of them agree that during Wallerian degeneration there are important alterations in the metabolization of acetylcholine. Also, it may be emphasized that these biochemical changes in what the amount of acetylcholine is concerned are somewhat independent of the conduction of nerve impulses. Coppée and Bacq (1938) and Mc Intosh (1938 have demostrated that in the pre-ganglionic sympathetic fibers undergoing degeneration conduction persists during the period when most of the acetylcholine of the nerve fibers has disappeared. Rosenblueth and Dempsey (1939) and Lissák, Dempsey and Rosenblueth (1939) demostrated that motor-nerve fibers undergoing Wallerian degeneration conduct impulses although acetylcholine may be intensely reduced. Feldberg (1943) has indicated that a nerve undergoing degeneration looses first its ability to synthetize acetylcholine than its capacity to conduct impulses. It may be interesting to mention here the observation of Croxatto, Huidobro and Luco (1940). These authors studied the cholinesterase activity of the superior cervical ganglion in the cat and were able to demostrate a reduction of 40-60 % between 24 and 72 hours after the section of the preganglionic fibers. This period of time corresponds to that of synaptic block and to that of noticeable decrease in the contents of acetylcholine (Mc Intosh, 1938). This decrease does not go further and it stays at values around 20-40 % of the normal values. It would be pertinent to mention similar studies. Hellauer and Umrat (1939) have studied the contents of cholinesterase of nerves, in preparations in which neuromuscular transmission was still normal: the cholinesterase content presented normal values: but when neuromuscular block appeared, cholinesterase diminished and when nerve conduction failed, this decrease was even more marked. Sawyer (1946) found a 60 % reduction of cholinesterase in degenerating axons. Nachmansohn and Hoff (1944) found a similar reduction of the enzyme after deafferentation of the spinal cord. Feng and Ting (1938) have demonstrated that after severance of the sciatic nerve in the frog, the muscle cholinesterase diminished in those

portions which had nerve endings. This observation was confirmed later on by Couteaux and Nachmansohn (1938, 1940).

The process of Wallerian degeneration has been divided in three stages by Johnson, McNabb and Rossiter (1950): (1) From 0 to 8 days the axone is destroyed and myelin becomes fragmentary although it does not suffer any process of chemical desintegration. By the end of this period there is complete fragmentation of myelin, conduction fails and there is a loss of enzymatic activity. (2) From 8 to 32 days myelin suffers maximal chemical destruction, lipids disappear and cholesterol esther is formed. Stage (1) may occur in vitro but stage (2) only in vivo. (3) During this stage myelin disappears. It is also interesting to notice the observation of Burt, McNabb and Rossiter (1950). The total content of lipids decreased during the first 16 days, they remained constant from 16 to 48 days and then increased slowly. Even 144 days afterwards the total content of lipids was lower than normal. Neutral fat decreased rapidly during the first 16 days but it returned to normal after 48 days. Myelin lipids (cerebrocids, free cholesterol and sphyngomyelin) decreased during the first 32 days, remained constant up to 96 days and then they increased gradually. After 144 days the total content of lipids was of the order of 44 % of control values. Total and free cholesterol decreased during the first 32 days. Cholesterol esther which is absent from normal nerves, appeared during this period.

c) The failure to obtain post-synaptic responses after presynaptic stimulation happens during a period in which pre-synaptic axons (nerve endings included) still conduct. This failure is the final stage of transmission block which may be demonstrated some hours after the nerve section and that it progresses slowly afterwards. Therefore transmission failure is gradual and not sudden (Rosenblueth, 1950). Titeca (1935) has demonstrated that synaptic alterations appear before the nerve stops conducting. Lissák, Dempsey and Rosenblueth (1939) demonstrated that the different stages of neuromuscular transmission become gradually altered. Between 24 and 48 hours after nerve section stages 2 and 3 disappear, therefore when the nerve is stimulated at a frequency of 400 stimuli per second, there is only an initial twitch and tension is not further increased although stimulation is applied for a long period. There is also some alteration of the 4th stage of neuromuscular transmission, which is obtained by stimulating the nerve at a frequency of 60 stimuli per second. The 5th stage will not appear during this process. These defficiencies are progressive and are especially marked 3 days after the section of the nerve. After this period, transmission fails completely. The cessation of transmission is the final result of a gradual process. Rosenblueth (1950) has suggested the following: transmission failure is parallel to the decrease of acetylcholine content of nerves but it does not follow a parallel course to the decrease in amplitude of the nerve action potential. It may be accepted that transmission failure occurs during a period in which the pre-synaptic fibers still conduct. Eyzaguirre, Espildora and Luco (1952) have demonstrated recently that the electromyogram of muscle suffers several progressive alterations until the complete disappearence of the muscle spike potential. After 60 hours of degeneration

they were able to observe some potentials which ressemble the local potential of the neuromuscular synapses. It is especially interesting to note that these alterations progressed from the point of nerve entry into the muscle toward more distal regions. In other words the temporal course of Wallerian degeneration, in what neuromuscular synapses are concerned, would be conditioned by the quantum of degenerating axon, i. e., the longer the axon the slower would be the appearence of synaptic failure. Now if it is accepted that neuromuscular transmission is mediated by a chemical agent and that the mediator is acetylcholine, which may be metabolized by the nerve, it could be understood that a longer axon means a more potent metabolic machine and therefore a larger reservoir of acetylcholine. During the course of Wallerian degeneration it is quite possible that there exists an exhaustion of the mediator, phenomenon which would be faster in a short nerve.

Alterations of innervated structures

We shall analyze only two aspects: fibrillation and sensitization.

It is well known that a few days after severance of the nerve, skeletal muscle fibrillates and that the adequate stimulus for this maintained activity is originated in the end-plates (Hayes and Woolsey, 1942; Jarcho, Berman, Eyzaguirre and Lilienthal, 1951). Another important characteristic of the fibrillatory process has been shown by Sarah Tower, (1937). She has demonstrated that if the motoneurone is left intact but isolated, the corresponding muscle suffers some alterations by denervation (atrophy, etc.), but it does not fibrillate. When the motor nerve was cut fibrillation started a few days later. In other words, the inhibition that is normally acting on the end-plate by impeding the generation of impulses by the latter is essentially conditioned by the functioning of the motoneurone.

The increased sensitivity of the denervated muscle structure is an example of Cannon's Law of Denervation, which was ennunciated several years ago. We do not feel that it is necessary to review here any aspects of sensitization due to the fact that a splendid monography is available (Cannon and Rosenblueth, 1949).

Luco and Eyzaguirre (unpublished data) have studied the role played by a degenerating nerve on the onset of fibrillation and of sensitization of denervated muscles. They have been able to demonstrate that similarly to synaptic alterations the time of onset of fibrillation and of maximal sensitization to acetylcholine is conditioned by the length of the degenerating neurone. When a muscle segment, which was closer to the site of nerve section, was compared to another segment which by its anatomical disposition was further away from that site, fibrillation and sensitization to acetylcholine appeared earlier in the former. Furthermore a given segment may be denervated by sectioning the axon at different levels. When the section was carried out close to the muscle fibers, fibrillation and sensitization started earlier than if the nerve was severed at some distance and centrally from this point. In conclusion it may be said that synaptic disturbances after Wallerian degeneration, fibrillation

and sensitization would be conditioned by the length of the degenerating nerve.

These observations would tend to indicate that the neurone even in its portions more distal to the muscle has an influence on the effector. In other words, the control that the neurone exerts on the end-plate and on the normal sensitivity of the muscle structure is a function which depends on the integrity of the whole axon.

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FACTORS WHICH ACCELERATE OR DELAY THE APPARITION OF PANCREATIC DIABETES IN THE RAT

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AFTER removal of 95 % of the pancreas in the rat, the diabetic symptoms appear gradually one at a time, taking about 1 to 3 months for all of them to be present. This characteristic evolution which we described in 1944 (2) makes the subtotally pancreatectomized rat a valuable tool in the study of: a) the correlations, evolution and date of apparition of the symptoms, and, b) the factors which delay or accelerate the apparition of diabetes.

Generally speaking, in all types of experimental diabetes, hyperglycemia follows immediatelly the causal agent, e. g., hypophyseal and adrenal diabetes in 1 to 3 days and alloxanic and total pancreatectomy in a few hours. Diabetes due to subtotal pancreatectomy in the dog is the only one with an evolution comparable to that of the rat; but the results in the dog vary from one animal to the other because of the tendency of the remnant of the pancreas to hypertrophy in some animals and to atrophy in others operated under the same conditions. Furthermore, the rat has many well known advantages as a laboratory animal (easy handling and obtention, large number, low cost, controlled inbreeding, etc.), and, the same as in man, is omnivorous, which makes it very useful for the experimental study of diabetes.

DIFFERENT STAGES OF DIABETES IN THE 95 % PANCREATECTOMIZED RAT

After removal of 95 % of the pancreatic mass in the rat, following our technique (2), the symptoms appear one at a time from the operation until the death in cachexia, 7 to 11 months later. This peculiar evolution can be divided into stages which are characteristic and very easy to determine, separated by conventional limits.

Since 1944 we consider the following stages and limits:

1) *Unapparent diabetes* or *prediabetes*. Which goes from the operation to the apparition of post prandial glycosuria;
2) *Incipient diabetes*. Which goes from the post prandial glycosuria to the beginning of fasting hyperglucemia;

3) *Manifest diabetes.* Which goes from the last point to the death of the animal.

The duration of each of these stages depends on the moment of apparition of their respective landmarks: post prandial glycosuria, fasting hyperglycemia or time of death. That is why the duration of each stage may vary within certain limits and has only statistical value; nevertheless, when maintaining the same conditions, results are very regular.

The final stage or *manifest diabetes* goes from the onset of fasting hyperglycemia until the death of the animal and in it all the known

TABLE I

Characteristics and evolution of symptoms following removal of 95 % of the pancreas in the rat.

	Prediabetes	Incipient Diabetes	Confirmed Diabetes
Limits	From pancreatectomy to alimentary glycosuria	From alimentary glycosuria to fasting hyperglycemia	From fasting hyperglycemia to death
Approximate duration (months)	Up to 1	1 - 2	4 - 7
Symptoms of diabetes	Incubation	Initiation, gradually increasing	Full symptomatology
Fasting glycemia	Normal	Normal	Hyperglycemia
Post-prandial glycemia	Normal	High	High
Glucose tolerance curve	Normal or slightly high	Higher	Diabetic
Alimentary glycosuria	no	yes	yes
Liver and muscular glycogen	Increased	Increased	Decreased
Growth (Body weight)	Normal or excessive	Slowed	Decreased
Spontaneous activity	Increased	Increased	Increased except near the end
Cataract; renal lesions	no	no	yes
Adrenal weight	Decreased	Decreased	Increased
Other organs (weight)	Normal	Normal	Increased in kidneys; decreased in thyroid, thymus, spleen, liver, testes

symptoms of the disease are present. Cataract and renal lesions constitute characteristic features as shown by us ([8, 9]). As a rule the rats are in a fairly good condition and have a prolonged span of life.

In the first two stages, blood sugar values become lower than 150 mg per cent after 7 hours fasting. This fact permits a simple separation of them from the third stage. The *prediabetes* is delimited by the operation and the onset of the post prandial glycosuria, easy to determine. It lasts from 15 days to 1 month. The rat is in good condition and the body weight sometimes exceeds the normal. After glucose administration it is possible to observe disturbances in the carbohydrate metabolism: blood sugar tolerance curve higher than the normal ([4, 10]) and a remarkable increase in hepatic and muscle glycogen content ([4]). The adrenal weight decreases ([5]) and the islets of Langerhans show signs of hyperfunction ([1]).

The second or *incipient stage* lasts 1 to 2 months and goes from the onset of post prandial glycosuria to the fasting hyperglycemia. Its principal characteristic is the onset and development of the diabetic symptoms. Body weight increases only slightly in spite of the polyphagia; polyuria and polydipsia appear and increase; the rat increases its spontaneous movements, a phenomenon which precedes the onset of hyperglycemia ([21]). Carbohydrate metabolism disturbances are seen after glucose administration (high blood sugar tolerance curves; increase in hepatic and muscular glycogen content ([4])) and post prandial hyperglycemia and glycosuria. It is important to note the normal level of blood sugar after 7 hours of fasting. Autopsy reveals atrophy of the adrenals and the β cells of the islets show degranulation, etc. ([1]).

The date of apparition of fasting hyperglycemia and its modifying factors

The occurrence of hyperglycemia after 7 hours of fasting marks the beginning of manifest diabetes. From the operation time up to this moment, an interval of 1 to 3 months elapses comprehending the prediabetes and the incipient diabetes stages. The determination of the exact date at which the fasting hyperglycemia appears has been shown to be very useful for the study of the factors which accelerate or delay the beginning of manifest diabetes because of the: a) easy determination of the moment of apparition of the hyperglycemia, making routine blood analysis; b) characteristic duration of the previous phase of normoglycemia (1 to 3 months) which makes it particularly apt for the prolonged study of different agents, and, c) the good condition of the rats at this moment.

The agents which anticipate the onset of the fasting hyperglycemia or, in other words, accelerate the beginning of the manifest diabetes, may be considered *aggravating factors*. On the other hand, the agents which delay the onset of the fasting hyperglycemia or, it other words, postpone or impede the apparition of the manifest diabetes may be considered *protective factors* ([13, 17]). In the so called "protected rats" the β cells are found in good form.

Until now, three main groups of agents are known to be able to modify

the date of apparition of the fasting hyperglycemia; they are: degree of pancreatectomy, diet and several endocrine influences.

Degree of pancreatectomy: The higher the degree of pancreatectomy the more anticipated the date of onset of the fasting hyperglycemia. So, after removing 90 % of the rat's pancreas the onset is delayed to more than 6 months ([2, 8]); removing 95 % to 1 to 3 months; on the other hand, removing more than 95 % the onset occurs after only a few days, and after total removal the manifest diabetes starts immediately ([6]).

Diet: Houssay and Martínez studied in subtotally pancreatectomized rats the influence of the amount, quality and way of administration of the diet on the date of apparition of the fasting hyperglycemia ([15, 18]). The increase in the amount of food anticipates the date and the contrary happens when the amount is reduced. The anticipation is also obtained by giving a large amount of food in 1 dose a day; the same amount is ineffective when divided into several doses a day.

The type of diet has a striking effect even when it keeps the same caloric value. The onset of fasting hyperglycemia is anticipated with diets rich in suet or lard. The contrary is seen with diets rich in proteins or coconut oil which have a protective role. An intermediate action between these two groups is seen with a carbohydrate rich diet.

Prolonged glucose injections have a delaying action and are accompanied by hyperplasia and hypertrophy of the islets ([16]).

Influence of endocrine factors: Studies have been particularly directed to the testes, ovaries and thyroid.

The role of the *gonads* on the apparition of diabetes was first demonstrated in 1945 ([3]) observing rats of both sexes with subtotal removal of the pancreas maintained under the same conditions. Females showed a more delayed apparition of fasting hyperglycemia than males; in other words, females are much more resistant to diabetes than males. Table II shows the results obtained in the original experiments now abundantly confirmed.

TABLE II

Number of diabetic rats after 95 % pancreatectomy.

	Six months after operation		A year after operation	
Females	4/12	33 %	10/25	40 %
Males	13/16	80 %	15/17	88 %

Castration in males slightly delay the onset of fasting hyperglycemia and in females anticipate it although not enough for both to coincide ([11]). If the animals are castrated at birth, both males and females developed the fasting hyperglycemia at the same time ([8]) this fact showing the importance of age at which castration is performed.

A similar protection, demonstrated by the delay in the beginning of diabetes, is also observed by injection of estrogens ([10]), natural and

synthetic *. On the contrary, male hormones accelerated the beginning of manifest diabetes. Through all these studies, we have reached to the conclusion that the gonads play an important role on the incidence of diabetes, which can be summarized by saying that the testes, through its hormones, favoured the apparition of the disease, and the ovaries, also through its hormones, protect the subtotally prancreatectomized rat against it.

The *thyroid* has an evident action on the apparition of diabetes, as was studied by Houssay, Martínez and other authors ([13, 14, 15]). Thyroidectomy performed at the same time that partial pancreatectomy in the rat is not followed by manifest diabetes during a year or more. Equal result is obtained giving thiouracil by mouth (table III). This "protection" disappears adding thyroid powder in the diet. All these experiments showed the important role of this gland on the apparition of diabetes in the rat.

TABLE III

Incidence of diabetes after 95 % pancreatectomy (data obtained from bibliographic References 13 and 14).

One year after operation			Six months after operation	
Pancreatectomy	Pancreatect. and Thyroidect.	Pancreatect., Thyroidect. and Thyroid Powder	Pancreatectomy	Pancreatect. and Thiouracil
95 %	16 %	86 %	92 %	60 %

SUMMARY

In the albino rat, fasting hyperglycemia appears only 1 to 3 months after removal of 95 % of the pancreas. The date of onset of fasting hyperglycemia is easy to determine and marks the end of two normoglycemic stages called *prediabetes*, without special symptomatology, and *incipient diabetes*, where symptoms appear and increase in severity. Following the onset of fasting hyperglycemia a stage of full symptomatology called *manifest diabetes* begins.

Different agents can modify the date of onset of fasting hyperglycemia or *manifest diabetes*. It is delayed by: 1) removing less than 95 % of the pancreas; 2) diet low in calories or rich in proteins; 3) estrogens, thyroidectomy and thiouracil. On the contrary it is anticipated by: 1) removing more than 95 % of the pancreatic mass; 2) diet rich in calories or in certain fats; 3) male hormones or thyroid powder.

Summing up: the rat to which 95 % of the pancreas has been removed is a very valuable tool, unique at this moment, for the study of factors which are able to accelerate or delay the apparition of diabetes or to modify the onset and development of the different symptoms.

* Oestrone; oestradiol; stilboestrol; ethynil-oestradiol; ethynil-testosterone.

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SLOW TONIC WAVES IN THE TURTLE HEART * (FANO PHENOMENON)

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IN PERFUSION experiments on the turtle heart, described in detail in a previous paper (14), rhythmic changes of auricular pressure and minute-volume were observed in numerous cases. Since the inflow pressure of the perfusion system was practically constant, irrespective of the cardiac resistance to flow, it was obvious that the systolic output of the heart was regulated by other factors beside the enddiastolic length of the fibers according to Starling's law. This anomalous behavior of the perfused turtle heart was the starting point of the observations which will be described in the present paper.

The above mentioned phenomenon has been known since 1887, when Fano (7) described for the first time slow tonic waves on the isolated turtle heart maintained in a NaCl solution. Records of the tonus changes of both atria showed that these waves were not synchronous, despite the normal beating of both auricles. Ventricular tonic waves were only registered 3 times in 100 observations. It was found later that several factors are capable of modifying the slow periodic activity of the turtle hearts. By increasing temperature the tonic waves were abolished, while the normal cardiac rhythm was maintained (7, 8). On the other hand, vagus stimulation causes standstill of the heart and increases markedly the tonic activity (7, 8). The "myogenic origin" of these waves was postulated by Fano, since the isolated auricles —without ganglia— showed both types of activity for as much as 24 hours (7). In seeking for an explanation of the tonus phenomenon, he studied the tonic activity of the turtle heart in relation to the changes of the demarcation current (Gaskell effect) on the auricular musculature (9). With an improved technique he was able to demonstrate the existence of a negative correlation between the tonus

* *This work was done in the Department of Physiology, Harvard Medical School, Boston, Mass., during the tenure of a W. K. Kellogg Foundation Fellowship. It is a pleasure to express my gratitude to Prof. Eugene M. Landis for his permanent interest in this work and for the facilities given in his laboratory.*

and membrane potential ([10]). Fano's original observations have been confirmed and extended by several authors (Botazzi ([2, 3]), Oinuma ([15]), Furuta ([12]), Grassmück ([13]), and others).

The influence of the cardiac nerves on the tonic activity of the turtle heart, particularly on the auricles, was studied by Botazzi ([2, 3]), who found that the stimulation of the sympathetic fibres causes disappearance of the tonic waves and increases the amplitude of systolic contractions; vagus nerve excitation had an opposite result. Electric stimulations of the vago-sympathicus causes first arrest of the heart and afterwards disappearance of the slow tonic waves. Grassmück ([13]) obtained similar effects.

Several agents are capable of increasing the slow tonic waves on the turtle heart, or inducing their appearance if they are absent ([8]). The addition of KCl, muscarine or digitalis to the perfusion fluid enhances the periodic changes of the heart tonus. On the contrary, atropine, nicotine, veratrine, papaverine and benzaldehyde are substances which reduce or even abolish these tonic waves. Polarization of the turtle heart with direct current, the auricles being connected to the positive pole, causes marked tonic activity ([11]).

In all the previous studies only mechanical recordings were obtained, both of cardiac rhythm and of the tonic changes of turtle hearts. No data is available on minute-volume and auricular pressure. The purpose of this study was to determine the correlation between the changes of auricular pressure and minute-volume caused by the slow tonic waves of the turtle heart.

METHODS

Common Cumberland terrapins (*Pseudemys scripta troosti*), weighing in the average 1200 g (range 950-1500 g) were used in perfusion experiments with a modified Ringer solution (NaCl 6.5 g, KCl 0.075 g, CaCl 0.2 g $NaHCO_3$ 0.2 g and dextrose 1.0 g in distilled water to make 1000 ml).

The perfusion system for the turtle heart has been described in detail in a previous paper ([14]). The rates of inflow were maintained constant at the desired level, irrespective of the height of the auricular pressures. The venous cannula (fig. 1) was introduced into the left pulmonary vein (LPVC); the arterial cannula into the aorta (AC). All remaining arteries and veins were tied off. Care was taken to avoid leakage of fluid via smaller vessels. A special large cannula was introduced into the right auricular cavity (RAC) and tied gently to the auricular wall.

Figure 1 (frontal view) shows the recording system. The perfusion fluid leaves the heart through the aortic cannula (AC) and reaches the outflow tube whose orifice was fixed at a constant height (arterial pressure). All pressures were measured with reference to the level of the heart (midpoint of the heart). The minute-volume was recorded by means of a rheometer (R), consisting of an U-tube with a capillary outflow resistance as shown in figure 1. One branch of the rheometer (R) was in front of the slit of a photokymograph (dashed line). A saline manometer

tube (M) was used to measure the right auricular pressure. Prior to each photographic record a few drops of diluted India ink were added to the saline in the rheometer (R) and in the auricular manometer (M). The time signal (1 per sec.) was given by a synchronous motor (T). A small lamp (S), in the lowermost portion of the slit, was used to indicate stimulation or injection.

Figure 1 (lateral view) shows the optical system, which consists of a lamp (F) and a biconvex lens (L) which illuminate uniformly the sensi-

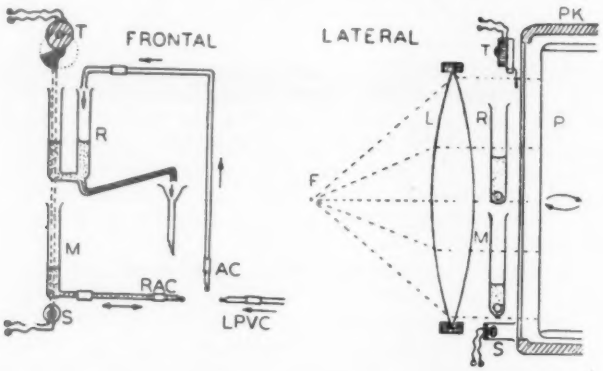

FIG. 1. — *a) Frontal view of the recording system.*
AC = aortic cannula; LPVC = left pulmonary vein cannula; RAC = right auricular cannula; R = rheometer; M = auricular manometer; T = time signal; S = signal lamp.
b) Lateral view of the optical system.
For description see text.

tive paper (P) of the photokymograph (PK). The tubes of the rheometer (R) and auricular manometer (M) were located between the lens (L) and the slit of the photokymograph. The time-marking device (T) and the signal lamp (S) are placed at the two extremes of the slit.

A total of 35 records from 16 perfusion experiments were obtained. Room temperature ranged from 21° to 25° C.

RESULTS

A record (fig. 2) of right auricular pressures of the perfused turtle heart shows that the intensity of auricular contractions changes from systole to systole. In the present case the differences between the highest and lowest peak values are around 50 mm saline. In other instances even greater pressure changes were observed. The amplitude of these regular waves was mainly determined by the rate of inflow to the heart, which could be adjusted to the desired level (see above). The period of the slow tonic waves, measured in 52 determinations, gave a mean value of 50 ± 2.3 seconds (mean $\pm$ 1 standard deviation of the mean), Thus around 25 or 30 cardiac cycles corresponded to one slow rhythmic wave.

Simultaneous recordings of right auricular pressures and cardiac outputs show (fig. 2b) that while the auricular pressure is increasing the minute-volume is decreasing in an exactly opposite manner. The phase-shift of these two representative values of the heart's activity is independent of the amplitude of the waves. As can be observed in figure 2b, the reduction of the perfusion pressure, i. e. the rate of inflow to the heart, does not affect the frequency of the slow waves nor does it influence the opposite behavior of auricular pressure and minute-volume.

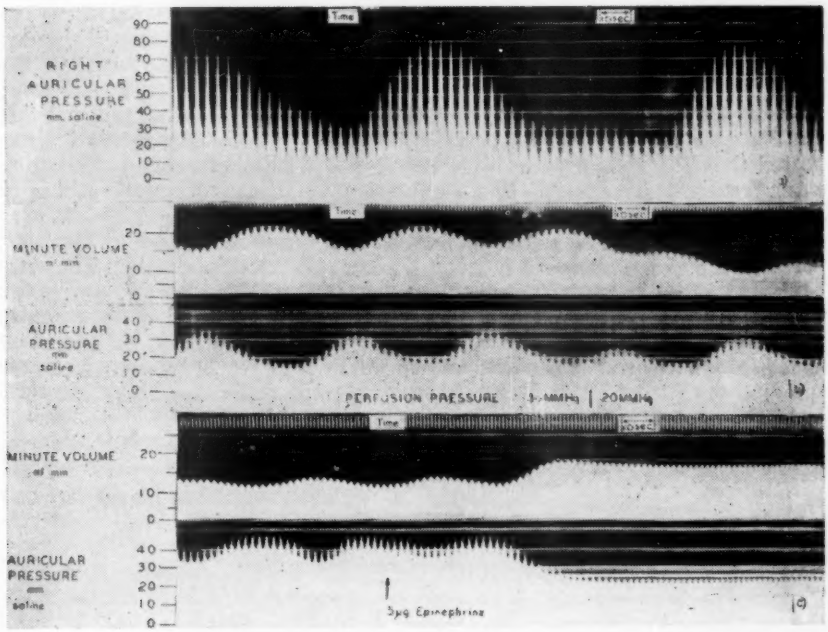

FIG. 2. — *Auricular pressures and minute-volumes of the perfused turtle heart.* a) right auricular pressure; b) minute-volume and right auricular pressure, after reducing the perfusion from 30 to 20 mm Hg; c) epinephrine effect (injection at the signal).

The tonic waves disappeared when epinephrine was added to the perfusion fluid by injecting the drug into the rubber tubing close to the venous cannula (fig. 2c). After a latent period right auricular pressure fell markedly while the minute-volume increased in a similar manner. Most noticeable was the disappearance of the slow periodic waves. The auricular pressure and the minute-volume became constant. Once the epinephrine effect was over, the tonic waves appeared again.

Several experiments with acetylcholine showed only a pronounced arrhythmia which interfered with the regular tonic waves of the heart.

Discussion

In the perfused heart an increase of auricular pressure is normally correlated —within certain limits— with a higher cardiac output. The heart of certain species of turtles seems to behave in a different manner; since as auricular pressure increases cardiac output decreases. This opposite relationship is more marked when the perfusion pressure is kept constant, as shown in this paper.

It is obvious that the rhythmic changes of auricular pressure and minute-volume can only be interpreted by assuming that the turtle heart changes periodically the intensity of ventricular systoles. Either the heart muscle itself is varying continously its contraction energy, or some extracardiac elements interfere with the dynamics of the turtle heart muscle. Rosenzweig ([17]) and later Bottazzi ([4]) and Oinuma ([15]), suggested that these slow tonic waves, observed with preference in the auricular tracings, may be due to the subendocardial smooth muscle fibres of the atria present in certain species of turtles. This hypothesis was confirmed by Shaner's ([18]) histologic studies and Chu's ([6]) control experiments. Shaner observed a complete smooth muscle coat in the atria (subendocardially) and radiating bundles of smooth muscle fibres in the ventricle. The veins, the sinus venosus and the arterial cone are also provided with smooth muscle cells, which are surrounded in all instances by a heavy sheat of collagen fibres. In a recent paper Robb ([16]) describes in detail the different varieties of muscular tissues in the heart, particularly the smooth muscle component.

The relative abundance of smooth muscle fibres in the auricles cannot explain *per se* the opposite variation of auricular pressure and cardiac output. We must assume that either a variable hindrance may exist between auricles and ventricle, or that the phenomenon is due to periodic changes of the systolic output. It is very likely, in view of the almost continous smooth muscle coat of the turtle heart's cavities, that both factors may be important. A contraction of smooth muscle fibres increases the resistance to flow in front of the A-V opening, as well as of the arterial cone and reduces the diastolic volume of the ventricle. The increased hindrance at the inflow and outflow orifices of the ventricle, together with a smaller enddiastolic volume, can explain the reduced volume output of the turtle heart during this period. Afterwards, a period of smooth muscle relaxation follows; the communication between the heart's cavities is widely open; the diastolic volume of the heart is increased; the heart muscle fibres are longer, and according to Starling's law the force of systolic contractions is enhanced. The consequence will be a greater minute-volume during the smooth muscle relaxation period. As the inflow to the heart is maintained practically constant by the perfusion system, the rhythmic variation of cardiac output must determine a concomitant change of auricular pressures. When the minute-volume is high the auricular pressure is low and viceversa.

The functional significance of the periodic changes of pressures and flows in the circulatory system of certain turtles is obscure. The internal smooth muscle coat of the heart may be considered as a reminiscence of its vascular origin. Thus the heart, located between venous and arterial systems, maintains a certain amount of primitive structural elements (smooth muscle cells). The other possibility is that these smooth muscle fibres play an important role in the regulation of the cardiac ouptput in certain species of turtles. In view of the fact that the chemical mediators of the vago-sympathicus nerves have marked influence on the tonic waves it is likely, that under certain circumtances the cardiac output can be varied within wide limits. The predominance of the vagus nerve leads to an increase of the tonic waves and to an inhibition of the heart's activity. Contrariwise, a sympathetic predominance, which causes smooth muscle relaxation, disappearance of the tonic waves, direct stimulation of the cardiac muscle fibres, will therefore produce a notable increase in cardiac output. Since cold has a similar effect to vagus stimulation in increasing tonic waves and on the other hand, heat and sympathetic stimulation have almost the same action, it can be assumed, that smooth muscle activity of the turtle heart may be an adaptation mechanism of the circulatory system to the seasonal metabolic requirements of these animals. In favor of this assumption are the observations of Rosenzweig ([17]) and Bottazzi ([4]), who found that the tonic waves are of higher intensity if the turtles were kept in a cold room prior to the experiment.

Despite the fact that tonic waves have been exclusively mentioned in relation to the isolated heart of certain turtles, occasionally periodic changes of tonus were found in the isolated rabbit heart during perfusion experiments with Ringer-Locke solution ([5]). This abnormal behavior persisted only for a short time (5-15 min). In other mammalian hearts slow tonic waves are not known. Nevertheless, even in the human heart several authors have described a subendocardial layer of smooth muscle fibres (see review of Benninghoff ([1])). They assume that these smooth muscle fibres are necessary for the adaptation of the endocardium to the volume changes of the heart during the cardiac cycle.

SUMMARY

In the perfused turtle heart slow periodic changes of auricular pressure and cardiac output were recorded. An increasing minute-volume was correlated with a decreasing auricular pessure and viceversa. After epinephrine, high cardiac output, low auricular pressure, and disappearance of slow tonic waves was observed. Smooth muscle components of the heart of certain turtles are responsible for the slow rhythmic tonus waves.

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ULTRAVIOLET ABSORPTION SPECTRA OF GENUINE OESTROGENS (INFLUENCE OF SOLVENT ON THE ABSORPTION SPECTRUM)

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MEASUREMENTS of the ultraviolet absorption of steroids have played a great role for the elucidation of their molecular structure. Jones (1948) has summarized the principles of ultraviolet spectrophotometry, and Friedgood and Garst (1948) have given a thorough description of the ultraviolet absorption spectra of the naturally occurring oestrogens: oestrone, oestradiol, and oestriol. The last-mentioned authors used the ultraviolet absorption measurements as a means of controlling the methods of extraction and partition of oestrogens in urine.

The absorption spectra of oestrone, oestradiol, and oestriol are almost identical, but as mentioned by Friedgood and Garst (1948) there is a small but significant difference in their molecular extinction coefficients, and the wavelength of the minimum absorption of oestradiol differs slightly from that of oestrone and oestriol. The general shape of the absorption curve is characterized by marked absorption beneath 235 mμ, minimal absorption in the neighbourhood of 250 mμ, an absorption band with maximum at 281 mμ, and practically no absorption at wavelengths above 300 mμ. Friedgood and Garst emphasize that there exists a secondary peak at 288 mμ in the curves for three oestrogens examined, and that this peak is shown in several of previously published figures by other authors, although not especially mentioned in the text.

Ethyl alcohol is the most commonly used solvent for ultraviolet spectrophotometry of oestrogens, and the description of the curves, given above, applies to ethyl alcoholic solutions. Ethyl alcohol was used as solvent by Butenandt and Störmer (1932), Mayneord and Roe (1937), Hogness *et al.* (1937), Heard and Hoffman (1941), Friedgood and Garst (1948), and others.

Pedersen-Bjergaard and Schou (1935) examined the ultraviolet absorption of oestrone in 50 per cent alcohol, to which was added HCl or NaOH. They found that in alkaline solution the absorption maximum was shifted from 282 mμ to 295 mμ, presumably due to an enolization of

the carbonyl group at C 17. Callow (1936) confirmed the shifting of the absorption peak in alkaline 90 per cent alcohol, but the fact that oestradiol and oestriol showed the same change of the absorption maximum as oestrone, whereas the absorption of oestrone-3-methyl ether was uninfluenced by the addition of NaOH, made it more likely that the effect was due to salt formation at C 3, and not to enolization at C 17.

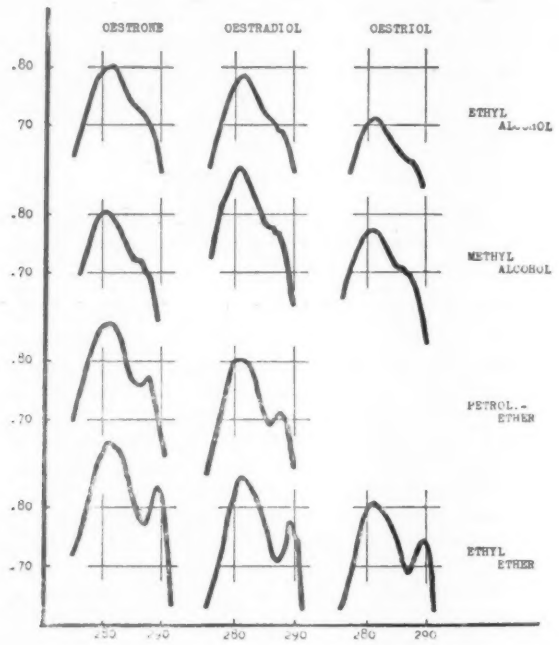

FIG. 1. — *Parts of ultraviolet absorption spectra of oestrone, oestradiol and oestriol in different organic solvents. Ordinates: D-values. Abscissae: wavelengths in* $m\mu$. *(Oestriol was insufficiently soluble in petrol-ether to allow the measurement).*

OWN INVESTIGATIONS

The purpose of the present experiments was to see, whether it was possible by changing the solvents to detect any differences of the absorption spectra of oestrone, oestradiol and oestriol. Using the Beckman Quartz Spectrophotometer Model DU and 1 cm. quartz cuvettes, the ultraviolet absorption of the oestrogens was measured in different solvents, viz. ethyl alcohol, methyl alcohol, petrol-ether, ethyl ether, 2 N aqueous NaOH, 0.3 mol. aqueous Na_2CO_3, glacial acetic acid, and 1 per cent acetone in ethyl alcohol.

The oestrogens used were: Oestrone (international standard prepa-

ration), Oestradiol (Ciba) (*), Oestriol (Parke, Davis & Co.) (*) and oestriol prepared in this laboratory from urine of pregnant women (melting point 276-278° C). Apropriate amounts of the steroids were measured out from stock solutions in ethyl alcohol and evaporated to

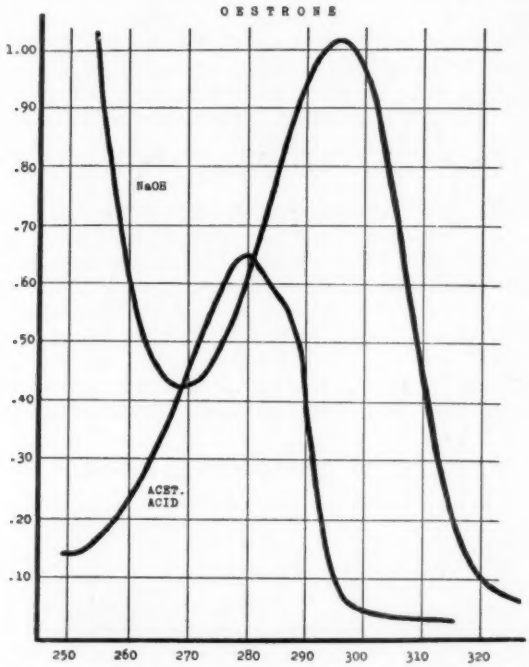

FIG. 2. — *Ultraviolet absorption spectra of oestrone dissolved in 2N aqueous NaOH and in glacial acetic acid. Ordinate: D-values. Abscissa: wavelength in mμ.*

dryness. *Solutions containing 100 microgrammes per ml. were used* for the present spectrophotometric determinations. The absorption was usually measured from 235 mμ to 320 mμ with 1 or 2 mμ intervals, and in certain regions with 0.5 mμ intervals.

The general shape of the absorption curves was the same for oestrone, oestradiol and oestriol. The curve for oestrone in methyl alcohol, shown in Fig. 3, may serve as an example: a minimum about 250 mμ, the peak of maximal absorption at 281 mμ, a secondary peak or "shoulder" about 288 mμ, and no absorption beyond 300 mμ.

Friedgood and Garst (1948) found *the lowest extinction* of oestrone and oestriol in ethyl alcohol at 248 mμ and that of oestradiol at 252 mμ.

(*) I am greatly indebted to *Ciba Limited*, Basle, Switzerland, and to *Parke, Davis & Co.*, Detroit, U.S.A. for generous supplies of these substances.

We have also observed such difference between oestrone and oestriol on the one hand and oestradiol on the other:

	Ethyl alcohol	Methyl alcohol	Ethyl ether
Oestrone	250	250	250-251
Oestradiol	254	253	253
Oestriol	249	249	248-249

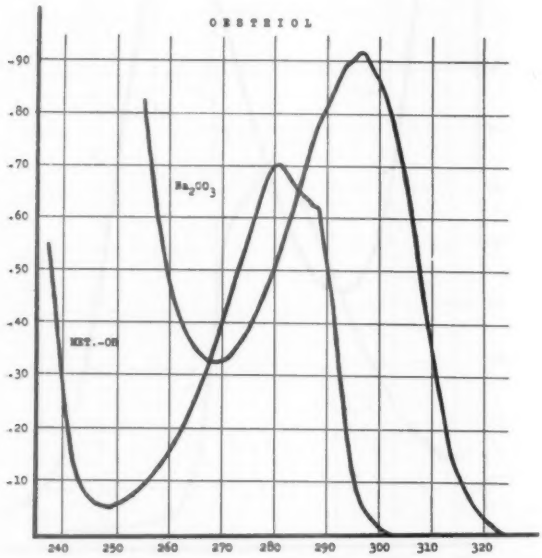

FIG. 3. — *Ultraviolet absorption spectra of oestriol dissolved in 0.3 mol. aqueous Na_2CO_3 and in methyl alcohol. Ordinate: D-values. Abscissa: wavelength in mμ.*

The secondary peak about 288 *mμ*, observed by Friedgood and Garst and other investigators, was in the present investigation found to vary according to the solvent used (see Fig. 1). This "shoulder" is inconspicuous in ethyl alcohol and in methyl alcohol; it is more marked in petrol-ether, and in ethyl ether the peak is very high and pointed. The exact position of this peak was 288 mμ in petrol-ether and 289.5 mμ in ethyl ether.

Examples of the *absorption curves in aqueous alkaline or acid solutions* are shown in Figs. 2 and 3. When the oestrogens were dissolved in 2 N NaOH or in 0.3 mol. Na_2CO_3, the peak of the absorption band was found at 296.5-297 mμ, and the minimal absorption occurred at 269-270 mμ. The absorption was much more intense than in organic neutral solvents. When the substances were dissolved in glacial acetic acid, the minimal and maximal absorption occurred at almost the same wavelengths

as in neutral organic solvents (Fig. 2), the peak of absorption being at 280 mμ.

It has not been possible by varying the solvent to detect any distinct differences between the ultraviolet absorption spectra of the three oestrogens. The shifting of the absorption bands towards the visible light in alkaline solutions is common for the oestrogens examined, and so was the accentuation of the secondary peak observed when ethyl ether was used as a solvent. It must be concluded that the peculiarities of the spectra are solely due to the nucleus of the molecule and independent of the carbonyl or hydroxyl groups at C 3, C 16 and C 17.

SUMMARY

Ultraviolet absorption spectra of oestrone, oestradiol and oestriol were measured in various organic and aqueous solvents.

The small peak or "shoulder" observed at 288 mμ by other investigators was found to be markedly accentuated, when the oestrogens were dissolved in ethyl ether.

The shifting of the spectra towards visible light in alkaline solutions was confirmed.

By changing the solvents no differences between the absorption spectra of oestrone, oestradiol and oestriol were observed.

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PROBLEMS OF REPRODUCTIVE HORMONE ACTION

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IN THE application of gonadotrophic and sex hormones to problems of animal production and fertility facts have emerged which seem to be of general physiological importance. It is the purpose of this paper to draw attention to two of these - the effect of hormones on the susceptibility of the uterus to infection, and the effects of the plane of nutrition of the animal on hormone action.

HORMONES AND UTERINE INFECTION

In cattle, and perhaps also in other species, infection of the uterus is probably the main cause of infertility (Quinlan, 1929). Much of this has been attributed to infection immediately after parturition and particularly in cases where involution and closure of the cervix does not occur quickly, and where some of the placenta is retained. The latter frequently occurs after cases of abortion in cattle, while in women the dangers of infection consequent on an induced abortion are well known.

The importance of hormone balance in such cases has been revealed by recent experiments in attempts to transplant fertilized ova via the cervix in cattle. In these experiments an attempt was made to insert the fertilized ovum through the cervix. This was done in the same way as is successfully practiced in the uterine insemination of cattle, but at the appropriate time in the cycle, namely, 4 to 5 days after oestrus and not, as in artificial insemination, at the time of oestrus (Dowling, 1949). Such attempts invariably failed and the cause for failure has been found to be due to infections set up in the uterus (Rowson, 1951; Brock and Rowson, 1952; Lamming and Rowson, 1952). Since in inseminations made at oestrus no such infection occurred, uterine inseminations were made from the same sample of bull semen into cows at oestrus and also into cows at 5 days post oestrus. While in the former conception and normal pregnancy occurred, in the latter infection of the uterus was invariably produced, the lumen containing much pus when the animals

were killed 2 days after insemination. Rowson, Lamming and Fry (1953a) then inserted cultures of bacteria into the uterus of cows in oestrus, and similar doses into cows at the 5th day after oestrus; both sets were slaughtered 2 days after the cultures had been inserted into the uterus. The uteri of the cows into which the culture was inserted at oestrus were sterile, but those innoculated in the luteal phase were invariably filled with pus from which the infective organism was isolated. This suggests that under the influence of oestrogen the uterine mucosa was resistant to infective organisms, whereas under the influence of progesterone it was extremely susceptible. To test this Rowson, Lamming and Fry (1953b) ovariectomized cows and into some injected stilboestrol and into others progesterone, and then into each set inserted a culture of bacteria into the uterus via the cervix. When killed 2 days later, the uterii of those injected with stilboestrol were sterile, while those injected with progesterone were filled with infected pus.

The next question to be answered was, how could infection of the uterus be prevented when an active corpus luteum was present in the ovaries and the uterus was under the influence of progesterone. Lipschütz (1924) was one of the first to investigate the quantitative antagonistic action of the reproductive hormones, and on the basis of his work and that of Marshall and Hammond Jnr. (1945) on the ferret where the oestrus-producing symptoms of an implant of stilboestrol could be overridden by implantations of progesterone, an attempt was made to see whether infection of the uterus of cows in the luteal phase of the cycle could be prevented by injections of stilboestrol. Lamming and Rowson (1953) found that this could be done; cows in the luteal phase of the cycle were injected by insertion of cultures into the uterus via the cervix, and a dose of 25, 50, 75, and 100 mg of stilboestrol was injected into a series of cows. As the dose of stilboestrol was increased the infection was reduced until at 100 mg it was overcome and the uterus was sterile.

The details of how these hormones work on the uterine mucosa to bring about this result have not yet been worked out, but there are many supporting facts for their conclusions and many further possibilities of the application of the results. For example, Laing and Day (1947) found that in Trichomonas foetus infection of the uterus in cows, many individuals made a spontaneous recovery if an oestrus period was allowed to elapse before mating, but if no oestrus period occurred they still remained infected. It has long been known (Marshall and Hammond, 1932) that squeezing out the corpus luteum per rectum in cows which were sterile increased their chances of fertility, this removal of the corpus luteum being one way of changing the oestrogen-progesterone ratio.

Further fields of work which are suggested by these conclusions are in the application of high doses of stilboestrol after abortions in which, owing to retention of parts of the placenta, the progesterone level is high and consequently the liability to infection great. There are possibilities also connected with infection in the male. While the cow, with its periodic periods of oestrus, can spontaneously recover from infection of Trichomonas foetus and Vibrio foetus owing to high levels of oestrogen at those times, no cases are known of recovery from infection of Tricho-

monas foetus in the bull. It is possible that this may be due to the fact that the mucosa of the bull is continually under the influence of testosterone, which has an action somewhat similar to progesterone. Would it be possible to override this action of testosterone by high doses of stilboestrol and so overcome the infection? At the time of writing, Rowson has obtained some indication that this can be done in the case of Vibrio foetus infections.

Effects of the Plane of Nutrition on Hormone Action

In the cow, a rising level of oestrogen about the 20th week of pregnancy causes growth of the alveolar cells of the mammary gland and the initiation of milk secretion. The implantation of tablets of stilboestrol under the skin of virgin heifers will cause the development of the cells and the secretion of milk within a few weeks (Hammond Jnr. and Day, 1944). During the course of these experiments it was noticed that much higher yields of milk from the same dose of stilboestrol were produced when the heifers were on a rising plane of nutrition, than when they were on a falling plane of nutrition. These observations were confirmed by Wallace (1948) in experiments on pregnant ewes, where, after the time when the oestrogen level became high (90 days pregnant), low plane nutrition resulted in only a small growth of the gland and yield of milk after parturition as compared with that of animals fed on a high plane of nutrition during this time.

Similar effects of the plane of nutrition on the results of gonadotrophic hormone injection on the ovaries have also been found. In the well nourished calf of a few weeks old Marden (1953) found that injection of horse anterior pituitary extract (APH) caused the growth of many large follicles, whereas in poorly nourished calves no effect could be obtained, even when very much larger doses of the hormone were injected. Adams (1953), too, working with this hormone in rabbits, found that animals reared on a high plane at about 12 weeks old reacted by ripening follicles, while litter mates reared on a low plane failed to do so, even though higher doses of the hormone were injected. The latter, however, after a week or two on high plane nutrition, although still much below normal in size, reacted well to the hormone by producing many large follicles.

There is supporting evidence for this conclusion that the plane of nutrition of the animal has a quantitative effect on the results obtained from the injection of a given dose of a hormone. For example, Underwood and Shier (1941) found that high plane as compared with low plane nutrition did not speed up the onset of the breeding season in sheep. That is, it did not increase the output of gonadotrophic hormone from the anterior pituitary. This increase in gonadotrophic hormone output from the anterior pituitary is caused rather by reflex reaction to increasing darkness-daylight ratios (Yeates, 1949; Hafez, 1952). Nichols (1926) and others have found, however, that increase in the plane of nutrition does increase the number of eggs shed at each heat period in sheep during the breeding season; that is, where the hormone is present in the blood stream

the plane of nutrition of the animal determines what quantitative effect it will have. From the results of these experiments it would seem that the quantitative results obtained from the injection of a hormone depend not only on the dose but also on the plane of nutrition of the animal into which it is injected.

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INFLUENCE OF THYROID HORMONE ON THE HEART-VAGUS SYSTEM

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IT HAS been shown that the lack or excess of thyroid hormone induces marked changes in the heart reactions to acetylcholine and adrenaline. Thus, the isolated heart from hyperthyroid animals shows less sensitivity to the depressor action of acetylcholine (Ach.) than the heart of normal animals; on the other hand, in hearts of hypothyroid animals, the reactivity to this substance appears to be increased. Sensitivity of isolated hearts of hyperthyroid animals to adrenaline (Adr.) is higher than in normal hearts while, in thyroid deficiency, opposite results are obtained (1).

Acetylcholine, as well as the stimulation of the vagi has, on the isolated mammalian heart, a positive chrono and inotropic action, that reveals itself at the end of the depressor effect, or during nerve stimulation, if atropine has been added to the perfusion fluid. Furthermore, the stimulating action of Ach or vagal stimulation has been shown to be in concomitance with the release of adrenalin-like substances (2).

In hearts of thyroxine treated animals, Ach. has a more intense stimulating effect than in normal hearts and, in the latter, this action is greater than in hearts of thyroidectomized animals (1).

This paper deals with some alterations of the cardioinhibitor and cardiostimulating action of the vagal system in conditions of experimental hypo and hyperthyroidism. Emphasis has been given to the sequence of events in heart rate in hyperthyroid animals, and alterations that occur in the vagus-heart system.

METHODS

Experiments were performed in isolated cats' hearts. The coronary vessels were perfused with Tyrode's solution saturated with gas mixture (O_2, 95 % and CO_2, 5 %) at 38° C. (Langendorff technic). An isotonic level was used to record ventricular activity.

The vagus nerves were dissected in the lower third of the neck and the thorax. Both nerves were stimulated simultaneously with 38/sec. alternative exponential impulses of supramaximal intensity.

Hypothyroidism was obtained: a) by thyroidectomy, 24-39 days prior to the experiment and b) by daily administration of 0.20-0.30 g of thiouracil "per os". Hyperthyroidism was induced by daily subcutaneous injections of thyroxine in dosis of 1 mg per kg throughout a period of 7-28 days.

Heart rate was checked every other day electrocardiographically. Experiments were done only on animals that showed fairly constant

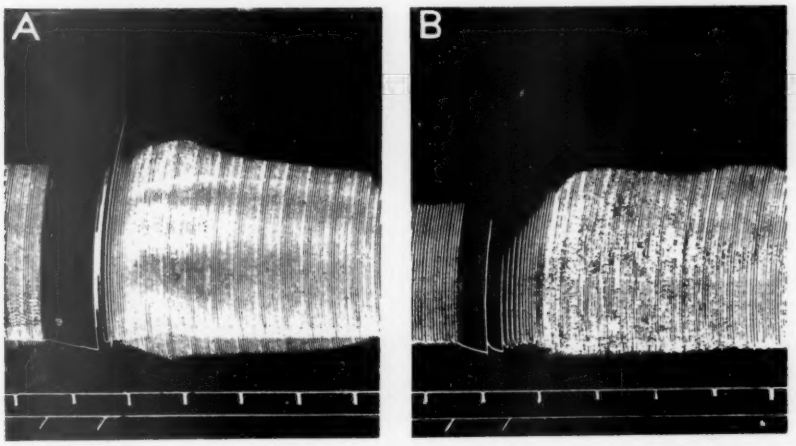

FIG. 1. — *Effect of the stimulation of the vagi on ventricular contractions of normal hearts.*
A. — Experiment V.T. 81. B. — Experiment V. T. 65. Time marks each 15 seconds. At the bottom vagus stimulation time.

heart frequency for at least 10 days before the beginning of the different treatments.

RESULTS

I. *Effect of vagal stimulation on hearts of untreated animals.* — From the 25 experiments of this group it can clearly be seen that 15 seconds of stimulation gives rise to heart stop followed by a variable period of increased frequency and amplitude of contractions. In the largest number of experiments the increased ventricular activity appeared at the end of the stimulation period. Two representative responses of this kind can be seen in Fig. 1. The enhanced heart activity that follows the cardiac arrest can be quantitatively expressed by the percentage of increase in rate and amplitude of contractions while the effect was at the peak compared with the activity of the ventricle immediately preceding the nerve excitation time.

In experiments A and B (Fig. 1) the frequency increase was about 40 and 80 per cent respectively, while the positive inotropic action was enlarged in both cases about 25 per cent.

II. *Effect of vagal stimulation on hearts of hyperthyroid cats.* — According to the length of time of exposure to thyroxine treatment, in this group of experiments, the cardiac action of the vagi was different.

Eight cats were treated less than 15 days, in two of these short time treated animals (7 and 9 days) no differences to vagal stimulation were noted compared with hearts of untreated cats. In the remaining six, that

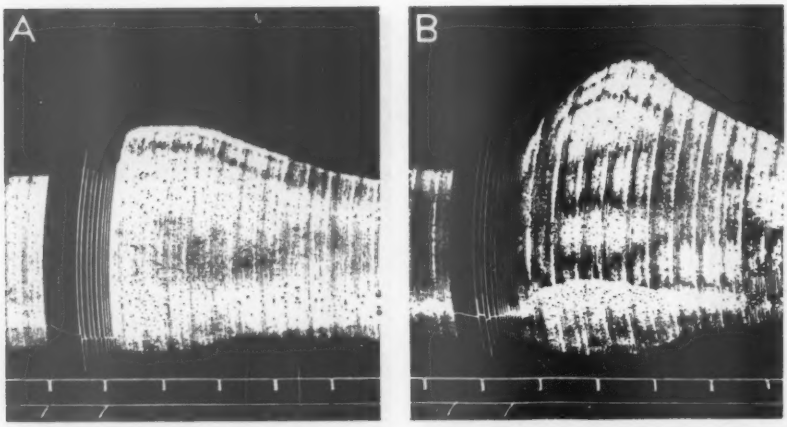

FIG. 2. — *Effect of stimulation of the vagi on ventricular contractions of hyperthyroid cats.*
A. — Experiment V. T. 76 (thyroid treatment for 14 days). B. — Experiment V. T. 58 (thyroid treatment for 24 days). Time marks as in Fig. 1.

were under treatment for 14 to 15 days, the cardioinhibition was of the same order as in the normal ones, but the following cardiostimulating fase was mainly emphasized by a rather explosive starting of the effect (A of Fig. 2). In this case the cardiac arrest is followed by an increase in heart rate and amplitude of about 40 and 25 % respectively.

Therefore, the prominent difference of the hearts of short time thyroxine treated cats, compared with the untreated ones, is that the cardiostimulating effect of the vagi appears suddenly instead of being gradual.

A characteristic record of an experiment on a heart of an animal maintained under thyroxine treatment for more than 18 days, is represented in B of Fig 2. It is noteworthy that vagus excitation induces a shorter stop of the ventricle and the enhancement in the systolic magnitude reaches about 75 %. This positive inotropic effect not only begins prematurely, is greater, but, in addition, lasts longer than in normal or short time hyperthyroid cases. In the same example of Fig. 2 the heart frequency increase was about 55 per cent, therefore, in the heart rate increase, the difference with the results on normal hearts is not significant.

III. *Effect of vagal stimulation in hearts of hypothyroid cats.* — a) In a few experiments with hearts of thyroidectomized animals, reactions were obtained very much alike to those described for normal hearts, as can be seen in B of Fig. 3. Nevertheless, in the greater number of cases, the cardioinhibitory fase induced by the vagi was emphasized, whilst the cardiostimulating fase was greatly reduced.

In A of Fig. 3 is shown that the vagus effect on a thyroidectomized animal's heart is characterized by cardiac arrest that persists for a time, after the vagal excitation, and that the recovery of amplitude and frequency is slow. Furthermore, the cardiostimulating fase is almost negligible

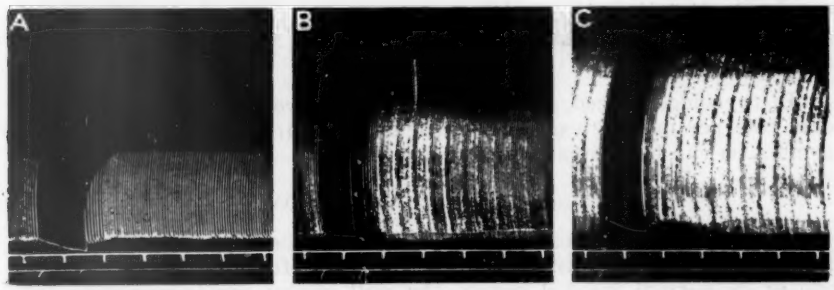

FIG. 3. — *Effect of stimulation of the vagi on ventricular contractions of hearts of hypothyroid cats.*
A. — Experiment V. T. 69 (thyroidectomy of 30 days). B. — Experiment V. T. 73 (thyroidectomy of 30 days). C. — Experiment V. T. 56 (thiouracil for 29 days). Time marks as in Fig. 1.

since frequency and amplitude of contractions were increased only about 15 and 7 per cent respectively.

b) In organs of thiouracil treated cats vagus actions were obtained like the one of C of Fig. 3. Nerve stimulation, in these cases, gave rise to marked cardiodepression with a very small cardioexcitatory fase; the heart frequency increased by only about 9 %, while no differences were recorded in the amplitude of contractions.

IV. *Heart rate changes in normal, hypothyroid and hyperthyroid cats.* — The average heart rate "in vivo" of normal animals was 145 ± 6 beats per minute. Once the heart was isolated the frequency diminished to a mean value of 116 ± 8 per minute.

The heart rate of thyroxine treated cats increased clearly after an average of two and a half days of treatment. Thus, when the experiments on the isolated heart were done, the short time hyperthyroid cats had an average of 256 ± 11 beats per minute and, the longer treated ones, an average of 230 ± 8 per minute.

During perfusion the frequency of both groups of hearts dropped to 172 ± 11 for the short treated and 153 ± 9 for the long treated animals. There is no significant difference between these values.

Though the frequency of the hearts of hyperthyroid animals decreased

when isolated from the body, an obvious difference persists compared with the isolated hearts of untreated animals, as can be seen in Table I.

TABLE I

Heart rate
(In beats per minute)

	In situ	Isolated
Thyroidectomized	175 ± 11	94 ± 13
Thiouracil	155 ± 9	105 ± 6
Normal	145 ± 6	116 ± 8
Short time thyroxine treated	256 ± 11	172 ± 11
Long time thyroxine treated	230 ± 8	153 ± 8

In thyroidectomized, as well as thiouracil treated cats, the average heart rate per minute was not significantly modified compared with the normals, as can be estimated in the same table.

Conclusions and discussion

In this paper previous communications have been confirmed, that supramaximal electrical stimulation of the vagi, for short time periods, induces, in normal isolated hearts, the well known cardiodepression followed by a fase of cardioexcitation that manifests itself at the end of the nerve stimulation. The records clearly show that the cardioexcitation involves heart rate as well as amplitude of contractions ([7]).

Vagal cardio-inhibition and stimulation are definitely altered by prolonged lack or excess of thyroid hormone. Vagal heart depression prevails in isolated hearts of thyroidectomized animals while the stimulating fase, that is almost unnoticed, manifests itself by a slight increase on heart frequency. The similar to normal responses obtained in some hearts of thyroidectomized animals could probably be explained by the assumption that in these few experiments hormonal compensation has been achieved by regeneration of thyroid tissue or aberrant gland or even by thyroid hormone like substance formed by extraglandular tissue. This assumption rests on the fact that in all thiouracil treated animals the heart reaction to vagal stimulation was uniformely characterized by prolonged cardiodepression with very little, if any, cardiostimulating effect.

Isolated hearts of more than 15 days thyroxine treated cats, reacted to vagal stimulation with reduced time of ventricular arrest in contrast with the ocurrence in hearts of short time thyroxine treatment. In addition, in long lasting hyperthyroidism it is noteworthy that the cardiostimulating vagal effect starts earlier, during the nerve excitation itself, or explosively at the very end of it. The cardiostimulating effect is more impressive on its inotropic aspect than on the heart rate. The percentage of increase of frequency lies in the range shown by normal hearts; nevertheless, it should be pointed out that the basic heart rate of the isolated heart of hyperthyroid cats was significantly greater than the corresponding values of the normal ones.

Many factors that are probably intimately correlated would play a role in the modified reactivity of the heart when thyroid treatment has been induced. The changes that the thyroid hormone brings about on the reactivity to Ach. and Adr. of some effectors ([3, 4]), particularly the heart ([1]), may explain, at least partially, the differences in magnitude of the cardio-inhibition by the vagus in hearts of hypothyroid, normal and hyperthyroid cats. The intensity of the cardiostimulating vagal action could be related to accompanying deviations of heart reactivity to adrenalin in conditions of lack or excess of thyroid hormone ([1]). In addition, it has been shown that by Ach., the release of adrenaline-like substances is greater in the isolated heart of hyperthyroid animals in comparison with normal or hypothyroid ones ([1]).

The studies on the mechanism of "vagal escape" in the isolated heart ([6]) have shown that the cardio-inhibition, by vagal stimulation, affects principally the excito-conductor system. During sinusal inhibition the ventricle escapes with a slower rythm by displacement of the pacemaker to a nodal or infra nodal portion. In hearts of hyperthyroid animals, this "cardiac escape" starts earlier; this fact may indicate that in hyperthyroidism the automatic property of infrasinusal portions of the cardiovector system is raised. On the other hand, in hypothyroid conditions the metabolic depression of the excitoconductor system could be responsible for the subordination of the myocardium rhythm to the pacemaker of the sinus node.

It is not known yet in what degree the thyroid hormone influences ganglionic transmission in the vagus nerve. Nevertheless, if interneural transmission is cholinergic, for the depressor as well as for the cardiostimulating nerve fibres ([2, 7, 9]), it has to be assumed that, in the hyperthyroid state, neural transmission may work at a higher level of acetylcholine; thus, the relative desensitization to Ach. that occurs in this condition could, in this way, be compensated; although there is no direct evidence of this, the results of experiments with incubated minced brain ([10]) are in favour of the hypothesis that the thyroid hormone hastens acetylcholine syntesis.

Therefore, it seems clear enough that in experimental hypo and hyperthyroidism, vagus heart transmission and cardiovector systems, are both disturbed. If one takes into account the heart alterations induced by the thyroid hormone, a fair explanation of the changes of heart behaviour to vagus nerve stimulation could be obtained. Basic tachycardia in hyperthyroidism would essentially be due to intrinsic modification of the sinus node's rate of stimuli; but it is not quite clear how the described alterations interact to produce the tachycardia of hyperthyroidism.

It is well known that tachycardia can be obtained by thyroid administration when total heart denervation ([8]) has been accomplished.

The experiments described in this paper gather further evidence in support of the hypothesis that in the increased basic heart rate of the hyperthyroid state the influence of the heart nerves is less important than the alterations induced by the thyroid hormone on the heart itself, since the increased heart frequency persists after the organ of the hyperthyroid animal was isolated. This has been well established

by other authors ([5]). On the other hand, tachycardia is present much before any changes of heart reaction to vagal stimulation can be detected.

SUMMARY

Vagi stimulation was applied to isolated hearts of normal, hypo and hyperthyroid cats.

In hearts of normal cats, the vagus effect is characterized by cardiac arrest followed by cardiostimulation that starts at the end of the nerve stimulation time. The cardiostimulating action can be observed on the frequency as well as on the amplitude of contractions.

In hearts of long time thyroxine treated animals the cardiac arrest was shorter and the stimulating fase started during the vagus excitation. The latter action that was larger and lasted longer than in normal hearts, was due more to enhancement of systolic amplitude than to heart frequency. The vagus effect on hearts of short time thyroxine treated animals was quite similar to the normal ones.

Electrocardiographic records of heart rate in intact cats showed that the tachycardia developed under short and long thyroxine treatments, was of the same order.

Once the hearts were isolated, the frequency was slowed down, but the differences persisted between heart rate of hyperthyroid, normal and hypothyroid animals.

In experimental long lasting hypo and hyperthyroidism the vagus heart system is disturbed, but this alteration does not explain, by itself, basic tachycardia in hyperthyroidism.

It is forwarded, in the discussion, that the alterations induced on the cardiovector system are the main factors involved in the changes of basic heart rate in hypo and hyperthyroidism.

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ADRENAL TUMORS IN GONADECTOMIZED RATS *

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ADRENAL cortical tumors have been shown to develop after the removal of the gonads in the guinea pig [17], many strains of mice [21, 23, 6, 16], the hamster [12, 20], and the rat [7, 8, 9, 10]. They are exceptional in non-castrated rats or mice.

In certain strains of mice these tumors are produced in 100 % of the castrated animals [24,25] whereas in other strains gonadectomy does not cause any adrenal tumor. According to the strain of mice either nodular or diffuse hyperplasia or adenoma or carcinoma may be found. Summaries of the studies on these tumors have been published [18, 19].

One strain of mice (NH) develops spontaneously adrenal tumors, when the animals are more than one year old and their gonadal function declines [5, 15, 4].

The adrenal tumors have estrogenic or androgenic actions. The carcinomas are transplantable to mice of the same strains and the grafts also produce estrogenic or androgenic effects [27].

EXPERIMENTS IN RATS

Experiments upon the appearance of adrenal cortical tumors in rats after the removal of their gonads have been performed in this Institute since 1948 on, and are being continued. We have studied 817 rats of both sexes in 15 experiments. The histological study of 498 adrenals has been already made.

Influence of adrenalectomy. — After bilateral adrenalectomy, accesory adrenals (average weight: 36 mg) developed in the 9 rats that

(*) This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

(**) Ciba Fellowship of the "Asociación Argentina para el Progreso de las Ciencias".

survived for more than 1 year. In 9 rats, in which both ovaries and both adrenals were removed, the accesory adrenals were smaller (average weight: 17 mg), because they lacked the trophic action of the ovaries. There were no tumoral or pretumoral modifications in the accesory adrenals of both experiments though the animals lived for 434 to 437 days after the operations (10).

In another experiment, one adrenal gland, grafted upon an adrenalectomized animal, developed an estrogenic adenoma one year after the operation.

Unilateral adrenalectomy did not produce adrenal tumors in castrated rats.

Technique of ovariectomy. — The technique of ovariectomy is very important. When removing ovaries with forceps ovarian regeneration (from small fragments of *rete ovarii or ovarian hilus*) with oestrus occurred in 13 % of the animals. For that reason, later on the ovaries and ⅓ or ½ of the uteri were removed with scissors and in that way ovarian regeneration was prevented (9).

In the rats with ovarian regeneration it was found that a) the oestrus disappeared after the removal of the ovarian fragment, b) it was intensified, after the removal of the adrenals, c) it disappeared after the removal of the pituitary and was restored by injections of gonadotrophins. These injections had no effect if the ovarian fragment had been previously removed (10).

Castration. — Noncastrated or unilaterally castrated rats did not show any pretumoral or tumoral modification of the adrenals.

Castrated rats had tumors in the adrenal cortices with high frequency (see table I). Their incidence was greater in females than in males for 415 days following castration (10). This sexual difference was smaller in rats castrated on being born (8).

Whenever the castration was performed either on the day they were born or in inmature or in adult rats, adrenal alterations appeared provided they lived long enough.

The adrenal modifications took place in the following order: they began with *pretumoral changes*, a) focal hyperplasias in the glomerular layer, b) cuneiform nodular hyperplasia, and later *tumoral changes* appeared c) adenomas (extracapsular or intraglandular), d) carcinoma *in situ* (8).

Foci of hyperplasia in the glomerular layer appeared shortly after operation, the most precocious one month after. Next, nodular hyperplasia developed generally after 5 or 7 months but the earliest ones after 2 months. Then they developed into adenomas, the earliest of which appeared after 8 months and a half, and their incidence increased gradually as time passed. Many adenomas elaborated estrogens, 12 to 16 months after gonadectomy, rarely sooner. These adenomatous adrenals were large, protruding, with an irregular surface, white or yellowish or sometimes reddish in colour, with some red or black spots owing to the dilated vessels. Abundant light coloured cells predominated in all the estrogenic adenomas.

TABLE I

Adrenal changes in bilaterally castrated rats

Experiments Nº	Days after castration	Rats Nº	Adrenal changes		Focal hyperplasia	Nodular hyperplasia	Adenoma	Carcinoma	Oestrus
			No	Yes					
Castrated females									
4 and 5	183-192	13	6	7	4	3	0	0	0
3	221	13	5	8	0	8	0	0	0
7(1)	240	3	2	1	1	0	0	0	0
6	270	17	6	11	2	4	5	0	0
7(1)	365	10	2	8	2	3	3	0	0
8	366	19	11	8	2	4	2	0	0
9	368	20	7	13	1	1	9	2	6
10	426	23	12	11	1	0	9	1	8
1 and 2(1)	415-437	34	?	?	?	1	8	0	9
7(1)	447	5	0	5	0	0	5	0	—
7(1)	730	3	0	3	0	0	3	0	—
Castrated males									
7(1)	240	5	2	3	1	2	0	0	—
7(1)	365	12	7	8	0	6	2	0	—
2(2)	415	22	20	2	0	0	2	0	—
7(1)	447	6	2	4	1	1	2	0	—
7(1)	730	3	0	3	0	2	1	0	—

(1) Castrated on the day they were born.
(2) The adrenals without visible tumors were not examined histologically.

The carcinomas *in situ*, were rarely found, only in 5 of the 498 adrenals and in some small foci.

In table I, only the more advanced pathological changes in the adrenals of every animal are shown, for different changes may often exist together in the same or both adrenals.

Estrogenic action. — When the adrenocortical adenomas elaborated estrogenic hormones, the vaginas, uteri, pituitaries and adrenals showed characteristic changes. The mammary glands have not yet been studied.

The vaginal smears first showed an increased number of leucocytes, then nucleated cells and finally only cornified cells. Mixed types of smears were also seen. At the beginning regular or irregular cycles were seen, but later on oestrus was produced continuously or for long periods. The typical images of oestrus were seen in histological sections of the vaginas. Only in 2 cases the vaginal epithelium was mucified.

The atrophic uteri of the castrated rats were stimulated by the estrogens and they were smaller, the same size or bigger than the normal ones. Hydrometra was found in some cases and pyometra in a few ones. There was hyperplasia of all the layers, glandular hypertrophy and sometimes cornified metaplasia of the uterine epithelium. Whenever we found a big uterus in a castrated rat, there was oestrus in the vaginal smear and adenomas in the adrenal cortices.

The pituitary glands are increased in weight when oestrus has existed for some time. They usually weigh between 15 and 22 mg but sometimes only 12 mg. The castration cells decrease in number or disappear and the granular basophillic cells become more numerous and in some cases form clusters. Hyaline basophilic cells, as those described by Crooke, were seen in some cases; this diagnosis was confirmed by Crooke himself, to whom some sections were shown. Similar changes have been found in mice ([2, 19]), but Crooke's cells were not identified.

In rats castrated on the day they were born pituitary tumors developed after 1 year and a half (2 adenomas and 2 carcinomas) and after 2 years (1 carcinoma in 3 male pituitaries and 1 carcinoma in 3 female pituitaries). Carcinoma diagnosis was histological. Tumor was not invasive and it was not transplanted.

Mechanism of the estrogenic action. — It was found that the corticoadrenal tumors elaborated the estrogenic hormones, for oestrus disappeared after adrenal removal and was not restored by injections of whole pituitary glands, gonadotrophins or adrenocorticotrophin ([8]).

Oestrus disappeared after removal of the pituitary gland in 2 to 4 days. Daily injections of adrenocorticotrophin did not restore it. The combined injections of chorionic and seric gonadotrophins (50 U.I. of each) restored it rapidly in all its intensity ([8]). The preventive action of hypophysectomy has been shown in mice ([13, 19]).

When the castrated rats were not in oestrus, we could neither produce it nor increase the frequency of adrenal tumors by injections of gonadothophins (3 weeks) or adrenocorticotrophin (83 days) though the size of the adrenals was increased by the latter ([8]).

Inhibition of adrenal tumors. — Some steroid hormones diminish or inhibit the development of the adrenal tumors that appear after gonadec-

tomy. Diethylstilbestrol inhibits the development of adrenal adenomas [14] or carcinomas [26] in mice. The same inhibitory effect is obtained by active estrogens, such as estradiol, estriol, and estrone, in mice [21, 14, 18, 19], estradiol in rats [1], and by active androgens [21]. Desoxycorticosterone acetate inhibits the development of adrenal carcinomas in castrated mice [11] and of adrenal adenomas in castrated rats [1].

COMMENT

Corticoadrenal tumors appear only after gonadectomy, but not if the pituitary is previously removed [3, 19]. If the pituitary is removed once the estrogenic tumors have developed, they stop secreting estrogens and decrease in size [8]. So they may be attributed to a pituitary action, that gland being altered by castration. Therefore there is a disturbed pituitary-gonadal relation.

Removal of the adrenal glands causes an increased secretion of pituitary adrenocorticotrophin, but this hormone is not able to produce any pretumoral or tumoral change in the accesory adrenals that develop in these rats.

Removal of the ovary stops secretion of estrogens and increases the production and secretion of pituitary gonadotrophins. There are probably some other disturbances in the secretion of pituitary hormones. It is not yet possible to explain how adrenal tumors are produced. Diminished amount of estrogenic hormones appears to be the *primum movens* of the adrenal changes.

At a certain moment corticoadrenal tumors become estrogenic and sensitive to gonadotrophins. The adrenal secretion of estrogenic hormones then comes under pituitary control.

Since in mice the results differ from one strain to another, we can accept the notion that genetic factors concur with endocrine ones in the production of adrenal tumors.

CONCLUSIONS

Adrenal cortical tumors develop in the white rat after gonadectomy, but much more slowly than in certain strains of mice. The adrenal modifications take place in the following order: a) focal hyperplasia, b) nodular hyperplasia (extracapsular or intraglandular), c) adenomas, d) very seldom carcinoma *in situ*. Many adenomas elaborate estrogens one year after castration. This estrogenic secretion is controlled by pituitary gonadotrophins. The appearance of these adrenal tumors is due to a disturbance of pituitary functions, caused by an alteration of the pituitary-gonadal hormonal balance.

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GLUTATHIONEMIA OF THE NORMAL RABBIT AND GUINEA-PIG, AND ITS VARIATIONS AFTER THE APPLICATION OF ALLOXAN

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IT IS KNOWN that alloxan combines with the SH group of the proteins; as the SH group is necessary for the activity of numerous ferments, and as it has been proved that various ferments are inactivated by alloxan and can be partially revived by adding glutathion whose molecule has an SH group, the investigations have continued, studying glutathion in relation to alloxan diabetes. Leach and Bailey (8) proved that the injection of glutathion to rabbits immediately prior to alloxan, prevents diabetes. They demonstrated, moreover, that the injection of alloxan to these animals, provokes an immediate descent of blood glutathion. De Caro and Rovida (3) communicated that after injecting alloxan to rats the hepatic glutathion descends to a 17 % of its original value, while the intestinal glutathion descends to 42 %.

Lazarow (7) corroborated Leach and Bailey's (8) experiments on the protective effect of glutathion injected before alloxan, and proved, besides, that adequate doses of glutathion or cysteine injected simultaneously or up to one minute after the alloxan injection have a partial protective effect.

It was observed also that a prolonged fast increased the sensitivity to alloxan and exerted an influence on glutathionemia.

It was necessary to study whether the resistance of the guinea-pig against the diabetogenic action of alloxan was related to the blood glutathion. Previously, the glutathionemia of rabbits and guinea-pigs under normal conditions was studied (11).

The blood glutathion was determined with the method described by Fujita and Numata (4, 5). The ascorbic acid was not destroyed previously, as it was present in a very minute quantity. We found very variable values of oxydized glutathion in dosifying reduced as well as total glutathion; for this reason we only took GSH into account, in our determinations.

We used 21 rabbits (1375 g to 2825 g) and 15 guinea-pigs (375 g to 500 g), males and females without discrimination, and fed them with

our usual laboratory diet. In six rabbits and three guinea-pigs the glutathion was determined while fasting, although receiving water in sufficient amount; the fasting lasted 51 hours for the rabbits and up to 47 hours for the guinea-pigs. 9 rabbits and 5 guinea-pigs were injected intravenously with 5 % alloxan (Eastman Kodak). The rabbits received 100 to 300 mg per kg and the guinea-pigs, 200 mg. The blood GSH of these animals was studied at different intervals, from half a minute up to one hour after the injection. The blood was obtained from the marginal vein of the ear, or by heart punction.

TABLE I

Protoc.	Sex	Weight G.	GSH	mg % (*)
2/62	M	1400–1800	52.2	(51.8 — 52.6)
3/56	F	1800	51.9	(50.7 — 53.0)
3/59	F	2100–2400	54.5	(53.7 — 55.2)
3/58	M	1375	54.3	(52.2 — 55.3)
3/57	M	1650	54.7	(52.3 — 56.0)
3/54	F	1650–1725	57.6	
3/49	M	1850	56.4	(56.0 — 56.8)
3/60	M	1525	51.0	(50.7 — 51.4)
2/67	F	1875–2825	50.7	(49.1 — 52.2)
2/0	F	1900–2450	53.7	(53.3 — 54.1)
2/29	M	2125	55.3	
AVERAGE			53.8	± 0.66
2/28	F	1650	39.7	(39.1 — 39.9)
2/26	M	1450	38.0	(36.8 — 38.4)
2/21	F	1800	35.1	(33.0 — 36.8)
2/10	F	2600	40.7	
2/13	F	2125–2225	35.3	(34.5 — 36.1)
2/14	F	1700–2200	34.8	(33.8 — 36.1)
2/15	F	2200	38.0	(36.8 — 39.1)
2/16	F	1600-2150	38.4	(37.6 — 39.9)
AVERAGE			37.5	± 0.78
Angora		1650	72.8	(67.5 — 77.9)

(*) This values are the average of 2 to 16 dosifications. The numbers between parenthesis are the highest and lowest value.

The values of the blood GSH obtained in our experiments in normal rabbits can be divided in two groups: one with an average of 53.82 mg % ± 0.68, and another with 37.47 mg % ± 0.78. The only rabbit of angora race which was used gave a blood GSH average of 72.76 mg % in 15 determinations. This value was not included in the average of the groups previously mentioned (Table 1). The average of the glutathionemia in the normal guinea-pig gave us a value of 57.93 mg % ± 0.78. Neither in the rabbit nor in the guinea-pig did the sex or the body weight show any influence on the glutathionemia.

In 4 animals there was no difference at all in the contents of GSH

between the arterial or venous blood obtained through heart punction, and not even prolonged fasting affected the glutathionemia of the six rabbits and three guinea-pigs.

The alloxan injection in rabbits produces an immediate descent of the blood glutathion which may reach O; but the values begin to recover

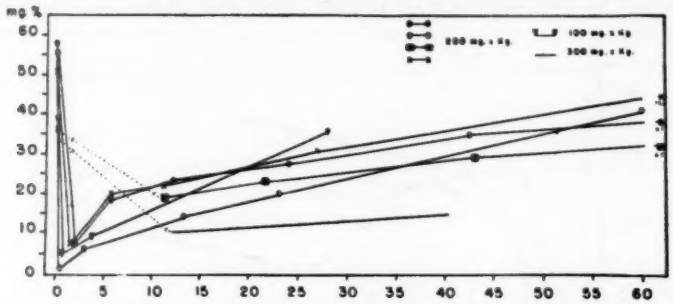

FIG. 1. — *Recovery curve of glutathionemia in normal rabbits after the intravenous injection of alloxan. Ordinates: reduced blood glutathion in mg %. Abscissae: time in minutes. The alloxan injection was made immediately after the O. The three dots after the 69 minutes, correspond, starting from top to bottom, to: 73.5, 65 and 65 minutes.*

rapidly. The speed of normalization is related to the doses injected; thus the rabbit which was injected with 100 mg per kg recovers its glutathionemia in 91 % of its original value in 28 minutes, while the rabbit which receives 300 mg of alloxan per kg recovers only 43 % in 40 minutes (Fig. 1). In general the values are still below the normal after 60 minutes. The type of the post alloxan glutathionemia curve does not depend on the initial amount of glutathion.

In the guinea-pigs under alloxan treatment we also observe the same brusque descent of the glutathionemia, but the values recover rapidly and they are almost normal after the first 10 - 20 minutes after the injection (Fig. 2). To demonstrate that the repeated heart punctions of the normal guinea-pig do not affect the values of the GSH due to hemoconcentration or other causes, a series of GSH dosifications was made in the normal guinea-pig, in a similar way as in those treated with alloxan. As no modification of the glutathionemia was observed, we do not insist upon this control.

DISCUSSION

Two groups of normal rabbits could clearly be distinguished: one with a high blood GSH (53.8 mg %) and the other with low values (37.4 mg %). Fujita and Numata's data ([4, 5]) agree with our low values. Collins-Williams, Renold and Marble ([2]) have obtained different values for their young and grown up rabbits. We saw that age (estimated by body weight) did not influence the distribution of the two groups. Moreover, the values obtained for the same animal, while young or grown up, were practically equal. The fact that in the only angora rabbit used, we

found very high values (72.7 mg %), and on the other hand the distribution of the GSH-values for the rabbits in two groups, induced us to think that the value of glutathionemia, fixed for each individual, may be genetically determined. The values found in the 15 normal guinea-pigs studied (57.9 mg %) are in accordance with those of Collins-Williams and col. (2).

As far as the influence of fasting over glutathionemia is concerned,

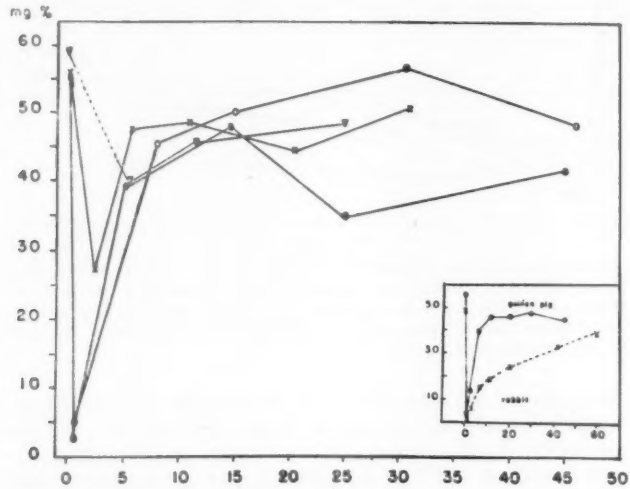

FIG. 2. — *Same as figure 1, but in guinea-pigs.*

our results prove the little or no effect of fasting of 47-51 hours on the blood glutathion. On the other hand, Leaf and Neuberger (10) demonstrated that hepatic glutathion of rats, descends greatly with a diet lacking proteins. These observation enable us to suppose the existence of a regulating mechanism which maintains the glutathionemia during fasting, at the expense primarily of the mobilization of the glutathion from the liver.

The endovenous alloxan injection produces in the rabbit a marked descent of the blood GSH, a fact which was already observed by Leach and Bailey (8). We found the tendency to reach O in the moments immediately following the injection, just as the latter authors proved it to be, but the recovery of the glutathionemia seems to be more rapid in our animals. The minimum value of 0.76 mg % found in rabbits, half a minute after the injection would correspond exactly to O, if we consider that with the technique used ascorbic acid was dosified as well.

Concerning the influence of alloxan on the guinea-pig's glutathionemia, our results prove that immediately following the alloxan injection the GSH also suffers an accentuated fall, but the minimum value obtained is higher than the one obtained in rabbits.

Comparing the recovery in both animals, we appreciate that the

guinea-pigs have a quicker tendency to recuperate their GSH, a fact which is clearly noticeable in the first 15 minutes following the injection.

The resistance which the guinea-pig offers to alloxan would be due according to Lazarow ([7]) to a greater quantity of glutathion present in the beta cells of the Langerhans islets. Besides this mechanism it is possible to explain the resistance through a compensatory action, probably of hepatic origin, which counteracts the momentary deficiency of glutathion in the beta cells, following the alloxan injection. Without denying the first possibility, we believe, according to our experimental results, that the second mechanism may be very important.

We can sum up the differences found between the guinea-pig and rabbit glutathionemia in the following points: 1. — The guinea-pig normally has a higher glutathionemia than the rabbit; 2. — The alloxan injection produces in it a lesser descent than in rabbits. 3. — With the same quantity of alloxan injected the recovery curve is faster in the guinea-pig.

SUMMARY

1) The average values of the reduced blood glutathion of 20 normal rabbits may be divided in two groups: one of 53.8 and another of 37.4 mg %. This difference bears no relation to the sex or the weight of the animal. In 15 normal guinea-pigs the average glutathionemia was of 57.93 mg %.

2) The glutathionemia of the normal rabbit and guinea-pig was not influenced by prolonged fasting.

3) The sudden descent of the glutathionemia, after the intravenous administration of alloxan is confirmed in the rabbit as well as in the guinea-pig.

4) The study of the recovery of the glutathionemia after the injection of alloxan shows a clear difference between the rabbit and the guinea-pig. While in the first the recovery is slow, being below the normal after 60 minutes, in the guinea-pig it is near the normal values after 10 to 20 minutes.

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INFLUENCE DES HORMONES SEXUELLES SUR LA PRODUCTION DU CANCER DU FOIE CHEZ LE RAT

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LES PROCÉDÉS de provocation de cancers, en particulier par les agents chimiques, ont mis à la disposition des chercheurs un matériel expérimental constant, permettant l'étude complète de quelques tumeurs déterminées. Parmi celles-ci, le cancer primitif du foie s'est montré particulièrement instructif. Les deux groupes de composés au moyen desquels on l'a obtenu avec le plus de fréquence sont: a) des azoïques, le premier découvert de ces cancérogènes ayant été l'ortho-aminoazotoluène, et le plus souvent employé le diméthylaminoazobenzène; b) des dérivés de l'aminofluorène.

Un des problèmes —entre beaucoup d'autres— qu'a permis d'aborder le cancer expérimental du foie est celui du rôle joué par le facteur sexuel dans sa pathogénie. Dans les pays où l'hépatome sévit avec une grande fréquence, on avait remarqué qu'il atteint les hommes beaucoup plus souvent que les femmes. Cette inégalité de prédisposition a été retrouvée expérimentalement par plusieurs auteurs.

TRAVAUX ANTÉRIEURS

Les premières expériences avec l'o-aminoazotoluène avaient déjà permis d'entrevoir une influence possible des sécrétions internes sur la production des tumeurs du foie chez le Rat. Tomita (1938), qui avait l'impression d'avoir hâté le développement des hépatomes par injections d'hormone mâle, obtenait un résultat semblable par l'orchiectomie (1940); tandis que Sukeharu (1939) avait constaté le même effet stimulant de la castration chez les femelles.

Utilisant l'acétyl-aminofluorène, Bielschowsky (1944), obtint des cancers du foie chez 34 rats mâles sur 41 (83 %), chez 11 femelles sur 36 (30 %), 5 sur 5 mâles castrés et 5 sur 11 femelles castrées (45 %). La différence sexuelle retrouvée par Leatham (1951), relativement aux tumeurs hépatiques produites par ce même toxique après six mois d'administration à des rats et des souris, était encore plus marquée: 90 % chez les mâles contre 10 % chez les femelles.

Cependant, par l'injection d'hormones sexuelles homologues, Cantarow, Pachkis, Stasney et Rothenberg (1946) parvenaient à augmenter

sensiblement le pourcentage des tumeurs kystiques et néoplasiques du foie. L'administration d'androgènes aux mâles accéléra leur apparition et leur évolution: l'injection d'oestrogènes aux femelles en fit passer le pourcentage de 55 à 92 %. Les auteurs conclurent que cette influence devait être en relation avec le rôle joué par le foie dans le métábolisme intermédiaire des hormones stéroïdes, ces dernières se comportant comme des cocarcinogènes.

Des faits du même ordre ont été enregistrés avec des azoïques. En 1951, Rumsfeld, Miller et Baumann firent connaître qu'utilisant le 3'-méthyl-4-diméthylaminoazobenzène, ils avaient constaté la plus grande susceptibilité des rats mâles qui, dans certaines expériences fournissaient 100 % de tumeurs hépatiques contre 20 % chez les femelles. La castration réduit cette inégalité (55 % chez les orchiectomisés et 44 % chez les ovariectomisées). Le facteur sexuel ne semble pas relever d'une différence du pouvoir détoxiquant de la cellule hépatique, puisque la destruction de l'azoïque *in vitro* s'obtient dans les mêmes proportions avec les tranches et homogénats de foies provenant de mâles ou de femelles.

Il importe de rapprocher ces faits de ceux rapportés dans diverses publications relatives a l'hépatome spontané, constaté chez quelques lignées de Souris. Tous les auteurs s'accordent pour reconnaître une plus grande fréquence chez les mâles. En revanche, les opinions divergent quant à l'action exercée chez ceux-ci, par les oestrogènes qui, pour les uns augmenteraient, pour d'autres diminueraient le pourcentage des tumeurs. Agnew et Gardner (1952), qui ont récemment revisé le problème, aboutissent aux conclusions suivantes: de fortes doses d'oestrogène empêchent l'apparition ou diminuent considérablement la fréquence de ces tumeurs chez les mâles; de même —mais de façon moins nette— la testostérone semble réduire la susceptibilité des femelles à produire l'hépatome.

Recherches personnelles

Dans l'étude du cancer expérimental du foie que nous poursuivons depuis quelques années, plusieurs expériences ont été consacrées au problème du rôle des hormones sexuelles dans sa production.

Chacun des lots équivalents, comprenant de 8 à 12 rats Wistar âgés de six mois environ, était constitué par des mâles ou des femelles entiers, des mâles ou des femelles castrés. Tous ces animaux recevaient pour nourriture des boulettes fabriquées au Laboratoire, représentant un régime complet mais pauvre en protéines et carencé en riboflavine, auquel était incorporé du diméthylaminoazobenzène synthétisé au Laboratoire (point de fusion: 115°) et dont la consommation quotidienne correspondait environ à 6 ou 8 mg. En outre, les rats des différents lots recevaient, par injections hebdomadaires, un des corps suivants: propionate de testostérone (3 mg), progestérone (3 mg), diéthylstilboestrol (50 γ), ou bromotriphynyléthylène (1.2 mg). Au total, 135 animaux ont servi dans ces expériences.

De grosses difficultés se présentent quand on essaye de trouver des éléments précis d'appréciation sur l'évolution des lésions hépatiques. Sur l'animal vivant, on ne dispose que de la palpation du foie (qui devrait être pratiquée sous anesthésie) et de la laparotomie exploratrice (avec

biopsie éventuelle). De telles explorations font courir des risques supplémentaires de mort prématurée à des animaux dont le régime alimentaire et l'absorption du toxique altèrent déjà sérieusement l'état général. La palpation ordinaire du foie a été généralement pratiquée chaque semaine (exceptionnellement sous anesthésie) ; la laparotomie (répétée à deux reprises chez quelques animaux) n'a été utilisée que sur trois lots.

Les seuls éléments de certitude de présence de tumeur maligne sont fournis après la mort de l'animal par l'autopsie (constatation de métastases) et par l'examen microscopique des prélèvements. Ceux-ci, à cause du décès inattendu et nocturne de beaucoup de rats, sont souvent fixés dans de mauvaises conditions de conservation. Cela ajoute à la difficulté du diagnostic histologique de malignité, qui n'est pas toujours aisé, même sur des coupes de foie obtenues dans les meilleures conditions. On s'explique que des auteurs parlent de tumeurs hépatiques sans indiquer s'il s'agit de cholangiectasies, d'adénomes, de cholangiocarcinomes ou d'hépatomes (la réalité de cette subdivision histopathologique étant d'ailleurs contestée par certains). D'autres précisent qu'il s'agit de tumeurs hépatiques malignes, mais sans autre précision.

Parmi les 135 animaux de nos expériences, 50 sont morts avant le 100ème jour. A l'examen histologique de leur foie, on a trouvé des degrés très variés de stéatose, de fibrose, de prolifération canaliculaire ; dans aucun cas on n'a constaté une transformation maligne certaine. Cependant, la palpation avait révélé la présence de tumeurs déjà perceptibles dans deux lots d'animaux recevant de la testostérone : au 90ème jour chez des mâles entiers, au 115ème jour chez des femelles castrées.

On a laparotomisé trois lots d'animaux : a) au 133ème jour, 8 mâles entiers, dont un seul présentait une tumeur macroscopique, alors que 6 de ces animaux devaient mourir de cancer avec métastases entre les 182ème et 277ème jours ; b) au 160ème jour, 9 femelles castrées et traitées à la testostérone, dont une seule atteinte de tumeur, alors que 5 devaient mourir de cancers aves métastases, du 195ème au 208ème jours ; c) au 193ème jour, 5 mâles castrés et traités au bromo-triphényléthylène, trois d'entre eux portaient une petite tumeur dont deux d'apparence maligne.

Ne pouvant entrer ici dans plus de détails, nous nous bornerons à fournir les résultats globaux de nos expériences comparatives, en fonction de deux éléments d'appréciation : la latence dans la manifestation des cancers hépatiques, leur fréquence dans chaque lot. Cela nous a conduit à classer ceux-ci en trois groupes : ceux où la cancérisation a été hâtée ; ceux où le taux de cancérisation peut être considéré comme normal dans les conditions de l'expérience ; ceux où ce taux a été manifestement abaissé.

Dans le premier groupe, tous les animaux ayant survécu plus de 100 jours sont morts de cancer du foie, entre les 128ème et 146ème jours. On y trouve, dans l'ordre de durée croissante de survie : les mâles simplement castrés ; les mâles entiers, ainsi que les femelles et les mâles castrés et traités à la testostérone ; les mâles castrés injectés à la progestérone.

Dans le second groupe, plus de 50 % des animaux sont morts de cancers hépatiques entre les 158ème et 303ème jours. Ce sont ; les femelles

entières; les femelles entières traitées à la testostérone; les mâles entiers; les femelles castrées.

Dans le dernier groupe, moins de 50 % des animaux sont morts de cancers du foie entre les 262ème et 308ème jours. Ce sont les mâles et les femelles castrés et injectés avec des oestrogènes.

En résumé, d'après ces résultats (dont certains divergent par comparaison avec ceux publiés par d'autres expérimentateurs), il semble que:

a) la castration des mâles augmente l'aptitude à la cancérisation (les pourcentages de Tomita (1940) et Bielschowsky (1944), l'avaient déjà indiqué), de même que l'administration de testostérone à des castrés des deux sexes, et celle de progestérone à des mâles castrés;

b) une différence dans la production de cancers hépatiques entre les mâles et les femelles non gonadectomisés n'est pas évidente;

c) l'administration d'oestrogène à des animaux castrés retarde l'apparition des tumeurs hépatiques malignes et diminue leur nombre.

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INFLUENCE OF SULFASUXIDINE ON THE EFFECT OF ALPHA LIPOIC OR THIOCTIC ACID ON THE VOLUNTARY ALCOHOL INTAKE OF RATS DEPLETED OF FACTOR N_1

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IN A PREVIOUS communication (1) we have reported that the administration of alpha lipoic or thioctic acid (cyclic disulfide derived from 6.8 dimercaptoöctanoic acid, that we will abreviate DTOA) to albino rats depleted of factor N_1, was followed, in about 50 % of them, by a significant decrease of the voluntary alcohol intake. This effect is not immediate. In fact the decrease of the voluntary alcohol intake was not significant during the first ten days of administration, and it was significant at the 1 % level during the period including the days 11th to 20th after starting the supplement, even when at this time the DTOA was discontinued. One explanation for this latency could be that this substance would need a previous elaboration that could be performed by the intestinal flora. To test this possibility we have studied the effect of DTOA given simultaneously with sulfasuxidine. The present paper reports the results of this study.

PROCEDURE

The experiments were performed on albino rats of our colony, following the same general procedure that has been reported with details elsewhere (2). Before starting the experiment the rats were fed for not less than 90 days with the diet lacking in factor N_1. The rats had free choice of 10 % V/V alcohol solution and of distilled water.

One group of 27 rats (11 males and 16 females) received a supplement of 2 % sulfasuxidine in the experimental diet. At the days 21st, 22nd and 24th after starting it, they received synthetic dl-DTOA orally in doses of 150 µg/100 g body weight/day. The sulfasuxidine was maintained till the end of the experiment. During the days 41st to 50th, the rats received a dry liver supplement in doses of 2g/100g body weight/day.

Another group made up of 13 rats (6 males and 7 females) were given the same doses of DTOA and of dry liver supplement at the same days than the group with sulfasuxidine.

The alcohol intake of each rat was measured daily and the difference of the arithmetic means of it during each period of 10 days was considered significant when $P < 0.01$.

RESULTS

Around two thirds of the rats employed in the experiment decrease the alcohol intake during the days in which they received liver sup-

TABLE I

Effect of liver supplement on the voluntary alcohol intake of rats depleted of factor N_1, with and without sulfasuxidine.

Conditions	Number of rats that change the alcohol intake at the 1 % level of significance after liver supplement	
	Decrease	No change
Sulfasuxidine	18	9
—	8	5

$\chi^2 = 0.09$ $P < 0.8$ and > 0.7

plement, i. e., only this proportion of rats are able to react to factor N_1. The data exhibited in Table I show that the proportion of rats that decrease the alcohol with liver supplement was similar in both experimental groups. On the other hand, this proportion is of the same order that the one observed in all our colony ([3]). That means that the decrease of the voluntary alcohol intake induced by the liver supplement was not affected by sulfasuxidine. Obviously we have to consider the effect of DTOA only in the rats whose voluntary alcohol intake was sensitive to liver. The results obtained on those liver sensitive rats belonging to each of the experimental groups are shown respectively in Tables II and III.

If we compare the results obtained during the third period in both groups we obtained a $\chi^2 = 0.63$, with $P < 0.50$ and > 0.30, meaning that the decrease of the voluntary alcohol intake observed in the group with sulfasuxidine is not statistically significant. On the other hand, the comparison of the results of the fifth period in both groups gave a $\chi^2 = 7.04$, $P < 0.01$. That means that the difference between the effects of DTOA in the presence and in the absence of sulfasuxidine is statistically significant. Since the group without sulfasuxidine could be considered too small, we can increase it with all the rats that have been tested in our laboratory under similar conditions with the same or lower doses of DTOA. In this way the group grows to 24 rats, 10 of them

TABLE II

Effect of sulfasuxidine and DTOA on the voluntary alcohol intake of liver sensitive rats depleted of factor N_1.

Period	Days	Supplements	Alcohol intake ml/100g body weight/day		Rats that decrease alcohol intake at 1 % level of significance (*)
			Range	*Mean and St. error*	
1	10	—	0.24 — 0.74	0.45 ± 0.032	—
2	10	Sulfa	0.23 — 0.67	0.43 ± 0.030	2/18 (1)
3	10	Sulfa	0.22 — 0.83	0.41 ± 0.040	3/18 (1)
4	10	Sulfa + DTOA	0.17 — 0.82	0.44 ± 0.045	1/18 (3)
5	10	Sulfa	0.15 — 0.69	0.38 ± 0.035	1/18 (3)

(*) The figure in parenthesis indicates the period with which it is compared.

TABLE III

Effect of DTOA on the voluntary alcohol intake of liver sensitive rats depleted of factor N_1.

Period	Days	Supplement	Alcohol intake ml/100g body weight/day		Rats that decrease alcohol intake at 1 % level of significance (*)
			Range	*Mean and St. error*	
1	10	—	0.23 — 0.63	0.42 ± 0.017	—
2	10	—	0.28 — 0.71	0.44 ± 0.044	0/8 (1)
3	10	—	0.21 — 0.84	0.43 ± 0.069	0/8 (1)
4	10	DTOA	0.30 — 0.56	0.37 ± 0.038	2/8 (3)
5	10	—	0.24 — 0.47	0.35 ± 0.028	4/8 (3)

(*) The figure in parenthesis indicates the period with which it is compared.

decrease the voluntary alcohol intake at the 1 % level of significance in the period including the 11th to the 20th days after starting the administration of DTOA. Then, the χ^2 became 5.84 with $P < 0.02$. In fact, this new study shows that when this group was increased the difference remained significant.

Discussion

The experimental results show that the effect of DTOA on the voluntary alcohol intake of rats depleted of factor N_1 was not observed when the substance was given simultaneously with sulfasuxidine; while the effect of liver supplement was not affected by this sulfadrug. This fact suggests that DTOA could not be itself active but it could be transformed into the active principle by the intestinal flora.

We are deeply indebted to Dr. E. L. R. Stokstad of Lederle Laboratories and to Dr. F. R. Van Abeele of E'li Lilly & Co. for the kind supply of the synthetic DTOA employed in these experiments.

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SOME ASPECTS OF THE MECHANISM OF BIOLOGICAL HYDROXYLATION OF STEROIDS

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IT IS KNOWN that enzymatic hydroxylation of steroids in 11-position takes place in the perfused isolated adrenal and liver, the perfused adrenal in situ, and under the influence of adrenal, hepatic or renal homogenates ([6, 7]) and also of fungus cultures of the genera Rhizopus, Streptomyces and Aspergillus ([6]). It has repeatedly been postulated that these reactions, and perhaps thus also the corresponding physiological conversions in the organism, may take place by way of dehydrogenation in ring C to 11:12 ([5, 9]) or 9:11 ([9, 3]) unsaturated steroids, and subsequent hydration of the resulting double bond.. An indication of this possibility was also provided by the fact that the addition of substances from the Krebs citric acid cycle —comprising an analogous reaction sequence from succinic into fumaric and malic acid (hydroxysuccinic acid)— proved necessary or at least very advantageous ([7]) when we worked with organ homogenates.

We were, in fact, able to demonstrate by paper chromatography the formation of 11β-hydroxyprogesterone, but also in addition that of the 12α-hydroxy compound, amongst others-([9]), upon treating 11-dehydroprogesterone ([8]) with adrenal homogenate under the conditions described earlier ([7]). This observation did not exclude entirely the possibility that the physiological hydroxylation proceeds also through 11:12-dehydrogenated compounds; the non-uniform reaction pathways, most probably also a result of using starting material of the progesterone instead of the adrenal cortical hormone series, did not however permit of any definite conclusions.

Following up our previous suggestion, we incubated aerobically with adrenal homogenate a 9:11-unsaturated compound of the adrenal hormone series, the Δ 4:5; 9:11 - 17:21 - dihydroxy - pregnadiene - 3:20 - dione - 21 - acetate (*).

(*) We are very grateful to Dr. Tishler of Merck & Co., Rahway N. J., for providing this 9:11-dehydro compound-S acetate.

In a preliminary experiment we were able to demonstrate by paper chromatography the formation of the corresponding products with hydroxyl groups in the 11β-position, i.e. the free 17-hydroxy-corticosterone and its 21-acetate.

Using a 75 mg batch of the above starting material, we thereupon succeeded in isolating 5 mg of 17-hydroxy-corticosterone acetate and 8 mg of 17-hydroxy-corticosterone, in addition to 50 mg of the unchanged starting material. The yield of the enzymatic 11β-hydroxylation thus amounts to about 53 % when allowing for the regenerated starting material. Other oxidation products, such as for instance cortisone or 11α-hydroxy compounds were not present in detectable amounts. It appears that the 11β-hydroxylation of the 9:11-unsaturated compound proceeds relatively uniformly and gives larger yields than that of compound-S acetate ([7]). With this material our earlier findings had failed to indicate direct hydroxylation without hydrolysis of the acetate, whereas in the present case of the 9:11-dehydro derivative, the 21-acetate can clearly be hydroxylated direct.

The reaction producing the 11β-hydroxylation here described strengthens our supposition that 9:11-dehydro-steroids represent important intermediates in the metabolism of the adrenal cortical hormones. It is interesting to see that these findings are complemented by those of Dobriner et al ([4]), who drew attention to disturbances in this metabolism in the course of neoplastic diseases, and especially to the significance of the excretion in urine of Δ 9.11-3α-hydroxy-5β-androstene-17-one (*).

Experimental

173 g of cattle adrenals obtained from freshly slaughtered animals were homogenised for six minutes in a mixer with 400 ml of a 0.04 molar fumaric acid solution containing 0.06 Mole potassium chloride, 0.02 Mole potassium phosphate, and 0.04 Mole magnesium sulfate per liter. The pH of the homogenate was held between 7.2 and 7.4 with N/1 potassium hydroxide solution. To one such batch we added 75 mg of 9:11-dehydro compound-S acetate (M.P. 233-9° C), to another 75 mg of compound-S acetate, while no steroid was added to a third. The mixtures were stirred for four hours at 37° C while oxygen was being piped in. The reaction solutions were thereupon brought to a pH of 4.5 with conc. hydrochloric acid, and each transferred into 4 lt of acetone. The acetone solutions were allowed to stand overnight, being stirred occasionally. They were then filtered under suction, the residue boiled up three times with acetone, using 200 ml each time, and the combined acetone solutions concentrated by evaporation. The residual aqueous solutions were extracted with ethyl acetate. The ethyl acetate extracts (2.5 to 3 g) obtained by evaporation under vacuum were dissolved in benzene and chromatographed on silica gel (Davison mesh thru 200). The extracts were eluted with benzene, a mixture of benzene and ethyl acetate, ethyl acetate, and methanol.

(*) However, this substance might be an artifact, produced from the 11β-hydroxy compound during acid hydrolysis.

The paper chromatogram (*) showed that the steroids were contained in the benzene-ethyl acetate (1:1) and ethyl acetate fractions. The control test showed only very slight traces of 17-hydroxy corticosterone. As expected, it was possible to demonstrate in the test with compound-S acetate the presence of 11β-hydroxy compound-S, besides some starting material and free compound-S. In the test using 9:11-dehydro compound-S acetate the paper chromatogram showed the presence of the starting material and both of its 11β-hydroxylated derivatives, compound-M acetate and also free compound-M. These steroid fractions (350 mg) were combined, redisolved in benzene, and chromatographed on 8.5 g of silica gel. They were successively eluted with benzene, methylene chloride, chloroform, mixtures of chloroform and acetone (99:1, 19:1, 9:1, 3:1, 1:1), acetone, and methanol. The partly crystalline residues of the various fractions were analysed by paper chromatography. The starting material itself (9:11-dehydro compound-S acetate) was eluted with chloroform, while the M-acetate was identified in chloroform-acetone (99:1) and compound-M in chloroform-acetone (3:1). The fractions mentioned were recrystallized from acetone-ether, and 5 mg 17-hydroxy-corticosterone acetate (M.P.209-18° C) and 8 mg 17-hydroxy-corticosterone (M.P. 212-20° C), were thus isolated, besides 50 mg of unchanged starting material. Paper chromatography established the uniformity of the crystals. When mixed with authentic samples of the corresponding substances, the products isolated showed no depression of the mixed melting point.

SUMMARY

The uniform conversion of 9:11-dehydro compound-S acetate into its 11β-hydroxylated derivatives (17-hydroxy-corticosterone and its acetate) by adrenal homogenate is demonstrated. This finding strengthens the supposition that 9:11-dehydro-steroids may represent important intermediates in the metabolism of the adrenal cortical hormones.

(*) We have to thank Dr. R. Neher (Ciba) for the paperchromatographic analysis.

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POSIBLE MECANISMO DE LA DEFIBRILACIÓN MIOCÁRDICA POR CONTRACHOQUE ELÉCTRICO

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SI BIEN desde fines del siglo pasado se sabe que la fibrilación ventricular puede ser abolida en el corazón por aplicación directa de corrientes eléctricas ([11, 12]), fué recién después de los experimentos de Hooker y colaboradores ([6]), de Ferris y colaboradores ([2]), y de Wiggers ([17, 18]), que se generalizó, como procedimiento experimental, la aplicación del contrachoque eléctrico para suprimir la fibrilación ventricular y obtener la reanudación de una actividad coordinada, capaz de restablecer una circulación sanguínea eficaz. Actualmente se recurre con gran éxito al contrachoque eléctrico para suprimir la fibrilación ventricular que puede producirse accidentalmente en corazones humanos en el curso de procedimientos exploratorios o quirúrgicos, siendo ya numerosos los casos de verdaderas resucitaciones que se han conseguido ([1]).

Llama la atención que hasta ahora no se haya intentado una explicación del mecanismo por el cual las corrientes de alto voltaje son capaces de suprimir la fibrilación. También es cierto que aún no existe acuerdo general acerca de la naturaleza íntima de la fibrilación miocárdica. Cualquiera sea el mecanismo que se considere en juego, ya sea el de una excitación irregular reentrante, según el concepto originariamente expuesto por Garrey ([4]), o el de una estimulación irregular repetitiva a partir de focos múltiples ([19]), o de un foco único ([13, 14]), todos los investigadores estarán problamente de acuerdo en que, esencialmente, la fibrilación es un tipo particular de actividad cardíaca caracterizado por la presencia simultánea y constantemente cambiante de fibras (o unidades funcionales) en contracción y de fibras (o unidades funcionales) en relajación.

En términos de excitabilidad, de acuerdo con lo clásicamente admitido, esto significa la prevalencia de una heterogeneidad absoluta en lo que a dicha propiedad se refiere, en las distintas fibras (o unidades funcionales) miocárdicas. En efecto, mientras las fibras en contracción estarán en fase refractaria absoluta, las fibras en relajación presentarán los distintos estados de recuperación característicos de la fase refractaria relativa o de la fase de excitabilidad completa. La figura 1 intenta re-

presentar en forma sobresimplificada, las condiciones de excitabilidad en 10 fibras miocárdicas (o unidades funcionales) contiguas, en un corazón fibrilado. La duración de los períodos refractarios absoluto y relativo se ha representado de acuerdo con lo encontrado en el corazón del perro por Orias y colaboradores (10).

Período refractario, excitación latente y desfibrilación. — En nuestra opinión una de las principales trabas que han actuado para que se eluda

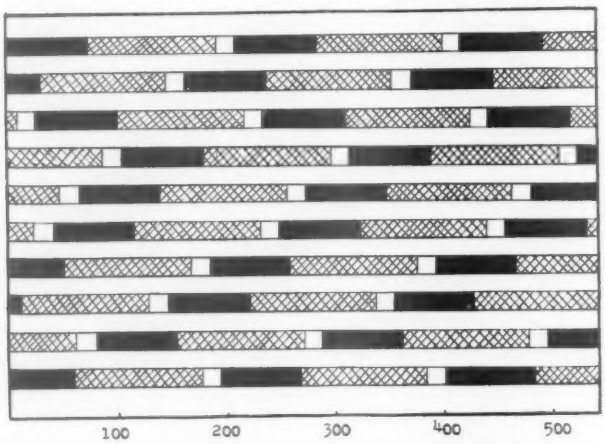

FIG. 1. — *Condiciones de excitabilidad durante la fibrilación miocárdica.* Cada franja horizontal representa la evolución de la excitabilidad en una fibra miocárdica. El sombreado compacto, indica el período refractario absoluto para un estímulo corriente de intensidad media; el cuadriculado diagonal, el período refractario relativo para el mismo estímulo y la porción no sombreada la fase de excitabilidad completa. El tiempo está marcado en milisegundos. El esquema muestra cómo en cada momento considerado hay fibras en cualquier estado de excitabilidad.

una explicación del mecanismo de la desfibrilación por contrachoque eléctrico la constituye, indudablemente, el concepto que tan rígida y arraigadamente prevalece acerca de la inexcitabilidad absoluta de la fibra muscular cardíaca durante su estado de contracción. Los conceptos de inexcitabilidad absoluta y de inexcitabilidad relativa han sido criticados con diverso fundamento (3, 9). Determinaciones de la excitabilidad cardíaca con procedimientos modernos han puesto en evidencia la falta de un período de inexcitabilidad absoluta en la fibra miocárdica del perro si se recurre a estímulos de duración e intensidad adecuadas (10).

De acuerdo con las ideas clásicas, una afirmación como la precedente inmediatamente sugiere la réplica de porqué, entonces, el corazón no puede ser tetanizado. La respuesta la dan los mismos estudios de la excita-

bilidad miocárdica a que se acaba de hacer referencia (10). En efecto, ellos también han puesto en evidencia que un estímulo que obre durante la fase de inexcitabilidad relativa nunca produce una respuesta inmediata, y que mientras más precozmente, durante dicha fase, obre el excitante, tanto más prolongado es el período de excitación latente (figura 2). O sea que si bien el músculo cardíaco puede responder a un estímulo aunque éste llegue aún al comienzo de una fase de contracción, es forzoso que

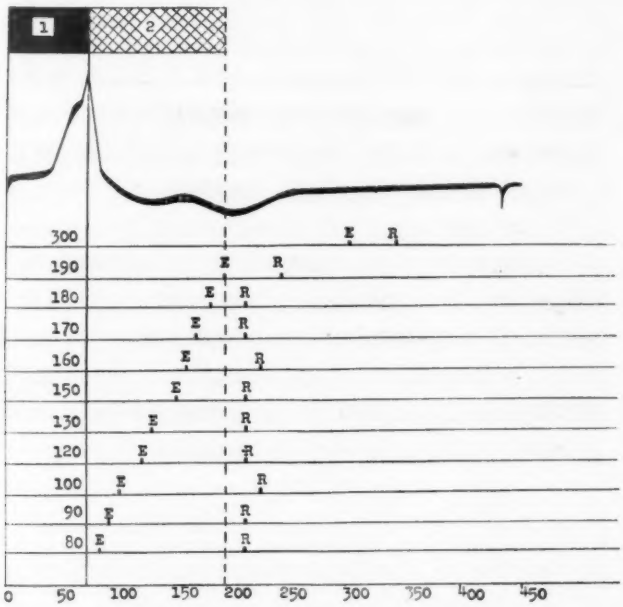

FIG. 2. — *Excitación latente y precocidad de estimulación.*
El trazado superior muestra el electrocardiograma de un latido provocado por un estímulo desencadenante aplicado directamente sobre el ventrículo en el comienzo de la escala del tiempo, marcada en el borde inferior de la figura en milisegundos. El sombreado compacto (1) muestra el período refractario absoluto para el tipo de estímulo empleado; y el cuadriculado diagonal (2) el período refractario relativo. E, marca el estímulo de prueba y R el instante de la respuesta. Los números marcados sobre las líneas horizontales corresponden al momento de aplicación, en milisegundos, del estímulo de prueba. (Orías y colaboradores (10)).

transcurra un cierto intervalo, suficiente para que se produzca por lo menos un esbozo de relajación, antes de que se instale el nuevo estado contráctil.

La comprobación de que una sola descarga de condensador puede abolir la fibrilación (11), apoya la idea de que es la eficacia excitante del estímulo en cualquier momento de la actividad de la fibra miocárdica la que posibilita esa eficacia. Más aún: la eficacia desfibrilante de un estí-

mulo único resulta una demostración concluyente de que produce su efecto (cualquiera sea la naturaleza de éste) en cualquier momento de la actividad de la fibra miocárdica. Si la desfibrilación se consigue mejor (con menor voltaje) con las corrientes alternadas, ello puede deberse a que los estímulos repetidos son más eficaces que los aislados (fenómeno de adición latente).

La figura 3 esquematiza el efecto de una corriente alternada de voltaje suficiente, de 60 ciclos por segundo, actuando durante 0.1 seg. sobre las mismas fibras representadas en la figura 1. Se da allí la idea de que

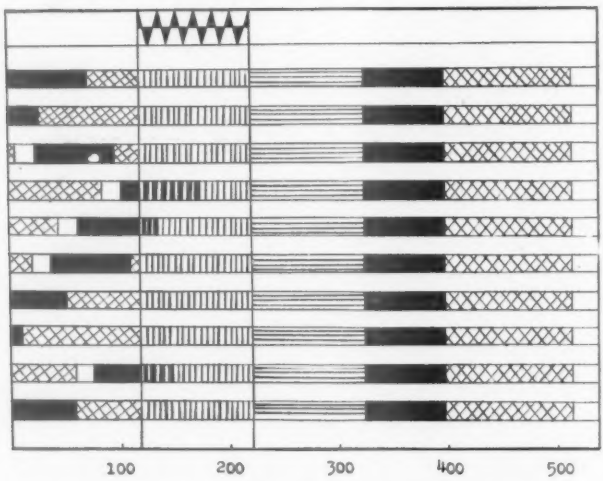

FIG. 3. — *Efecto desfibrilante de una corriente alternada.*
Sobre una situación idéntica a la representada en la figura 1, se ha esquematizado el efecto corrector de la fibrilación de una corriente alternada de voltaje suficiente. El sombreado vertical representa el estado de excitación creado y mantenido por los pulsos eléctricos, aún en las fibras que presentaban un estado refractario para estímulos corrientes y el sombreado horizontal representa el período de excitación latente.

la estimulación resulta eficaz para crear un estado de excitación (rayado vertical), aún en las fibras que serían refractarias para estímulos corrientes, el cual, después de un cierto período de latencia (rayado horizontal), produce una respuesta contráctil (sombreado compacto representativo de la fase refractaria absoluta para estímulos de intensidad corriente). De la simultaneidad de los estados de excitación y de las respuestas así obtenidas resulta el ordenamiento de la actividad de las fibras miocárdicas y, por ende, la abolición de la fibrilación.

Para ser eficaz el contrachoque eléctrico su efecto debe ejercerse sobre todas, absolutamente todas, las fibras miocárdicas. Mientras alguna o algunas de ellas escapen a él, persistirá la actividad desordenada en alguna porción del miocardio, e instantáneamente la incoordinación se

generalizará nuevamente a toda la masa. De esto resulta la necesidad de actuar con electrodos que abarquen la mayor superficie cardíaca, aplicados lo más cercanamente posible al corazón y el uso de elevados voltajes y altas intensidades ([5, 7, 8]).

Fundamento de la actividad cardíaca coordinada. — En definitiva puede inferirse que la persistencia de la fibrilación se debe a la persistencia de un estado de heterogeneidad en las condiciones de excitabilidad de las fibras miocárdicas. Para su abolición quedan dos posibilidades: a) llevar todas las fibras a un estado de relajación, o bien, b) llevar a todas las fibras en un instante dado, a un estado de contracción. En ambos casos se consigue la uniformidad de las condiciones de excitabilidad de las fibras miocárdicas, base indispensable para una actividad coordinada.

Por el primer mecanismo ejerce su acción desfibrilante el potasio ([18]). Por el segundo mecanismo actúa el contrachoque eléctrico.

RESUMEN

Sobre la base de que estudios experimentales del autor, realizados en colaboración con otros investigadores, han demostrado que si se usan estímulos eléctricos de duración e intensidad suficientes la fibra miocárdica puede responder en cualquier momento de su actividad (falta real de un período refractario absoluto), se explica la acción desfibrilante del contrachoque eléctrico por la creación de un estado de excitación simultáneo en todas las fibras miocárdicas, independiente del estado de contracción o de relajación en que cada una de ellas se encuentre en el momento de su aplicación.

SUMMARY

On the basis of experimental studies showing that if electric stimuli are used of sufficient intensity and duration, the myocardial fiber can respond at any moment of its activity (there is no real absolute rephractory phase) the defibrillatory effect of the electric counter shock is attributed to the establishment of a simultaneous excitatory state in all the myocardial fibers, regardless of their condition of contraction or relaxion at the moment of its application.

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ON THE POSSIBLE SHARE OF ANDROGENS IN THE DEVELOPMENT OF SECONDARY SEX ORGANS IN THE FEMALE RAT

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SINCE Lipschutz and his collaborators have always shown much interest in the effects of androgens in the female, and recently reported on oestrogen-androgen antagonism on the clitoris of the guinea pig (Bruzzone and Lipschutz, 1953), it is a pleasure to us to be able to contribute a paper to this special issue dealing with a related item.

Form, size and function of female sex organs have long been considered to be mainly determined by the "female" hormones oestradiol and progesterone. Later, a contribution to the development of ovarian follicles by androgen was made plausible a. o. by Gaarenstroom and de Jongh (1946): androgens stimulate antrum formation and ovulation. Paesi and Gaarenstroom (1943), showed that the origin of the androgen produced in hypophysectomized animals stimulated with chorionic gonadotrophin has to be sought in the interstitial tissue of the ovary.

Other autors (e. g. Korenchevsky and Dennison, 1936), brought arguments in favour of a possible role of androgen *outside* the ovary: in the normal development of secondary sex organs. Their opinion was shared by Burrows (1949), who writes on page 242: "Apparently the uterus, like other accessory sexual organs whether in the male or female, requires for its well-being a balanced supply of androgen and oestrogen".

There are, moreover, a number of phenomena, summarized by de Jongh (1951), which might easily be explained by accepting an androgen to take part in the moulding of the secondary sex characters of the female. Among these is the experience of one of us (P.) that oestradiol (benzoate) is unable to bring the weight of the spayed rat up to the normal without at the same time increasing the weights of hypophysis and adrenal glands to higher than normal values. An androgen might well be the missing link which helps to maintain normal weights since testosterone is known not only to be able to enlarge the uterus and to increase its oestrogen-induced weight gain (Korenschevsky and Dennison, 1936), but, moreover, to diminish the weight increase of the hypophysis obtainable with oestrogen (Wolfe and Hamilton, 1937; Gaarenstroom and de Jongh, 1941), and to decrease the weight of the adrenal (Burrows, page 271).

These considerations led us to start an extensive investigation into the possibility to restore the normal proportion between the weights of uterus, adrenals and hypophysis by administering various doses of testosterone propionate to oestrogen-treated spayed rats. The results (which were negative) will be published elsewhere but we want to stress one detail here which we learned from them: it is most probable that the maximum concentration of androgen to occur spontaneously in the female is less than that obtained by injecting as little as 4 μg of testosterone propionate daily. This would deprive the observations of Korenchevsky et al., however correct in themselves, of a physiological significance and limit the amount to be substituted to very small doses.

Methods

Adult spayed rats (approx. 200 g) were treated for 14-39 days with small doses of oestradiol benzoate (0.05-2.5 μg daily) in an oily solution (twice daily 0.1 ml). In a number of these animals the oestrogen was supplemented with 2-8 μg of testosterone propionate daily. Control rats received solvent only. In ten subsequent series alterations were made in the type of oestrogen (oestrone instead of oestradiol benzoate) as well as in the dosage, the solvent (water i. s. o. oil) and the injection scheme (intermittently i. s. o. continuously, as an imitation of the cycle). Since details about the mode of administration of oestrogen are of no importance for the problem at hand, they are omitted here. In table I each line represents one experimental series. The average clitoris weights are listed; these form the basis for our argumentation.

Results

The table shows a marked increase of the clitoris weight when 8 μg of testosterone propionate was administered together with oestrogen (i. c. oestrone, intermittently adm.). Even with as little as 4 μg of testosterone propionate (combined with oestrogen treatment) the clitoris reached a higher weight than in control animals treated with oestrogen only: the mean of the average increase per series amounts to 2.0 ± 0.63 mg, which is statistically significant. Four series also comprised normal nonspayed females and in all four the clitoris weighed less than that of testosterone (4 μg)-oestrogen treated spayed companions. The mean of the average differences is 3.5 ± 0.90 mg, which is also significant. The results suggest moreover, that oestrogen itself is able to enlarge the clitoris (diff.: 1.3 ± 0.57; not significant).

It should be stressed that in all the experiments mentioned testosterone propionete has been given *together with oestrogen*. Preliminary results show, however, that without oestrogen the same amounts of testosterone are also able to cause clitoris growth.

Discussion

With as little as 4 μg of testosterone propionate administered simultaneously with small doses of oestrogen an enlargement of the clitoris as

TABLE 1

Mean clitoris weights (mg) in groups of adult rats treated either with oestrogen or with oestrogen and testosterone propionate (2 μg, 3 μg, 4 μg, or 8 μg daily). Number of animals between parentheses. Each line contains data about one single experimental series.

Nr. of series	I Spayed control rats	diff. II - I	II Spayed rats oestrogen-treated	III	IV	V	VI	diff. V - II	VII Normal control rats	diff. V - VII
				Spayed, oestrogen-treated rats receiving testosterone propionate in addition in the following daily doses:						
				2 μg	3 μg	4 μg	8 μg			
1	10.5 (8)	3.0	13.5 (8)	12.5 (9)		12.5 (8)		—1.0		
2			14.5 (8)			17.7 (7)		3.2		
3	7.9 (4)	2.2	9.9 (10)			11.4 (10)		1.5		
4	10.0 (4)	1.0	11.0 (10)			11.8 (10)		0.8		
5	12.0 (3)	2.0	14.0 (4)			17.0 (3)		3.0		
6			12.0 (3)	14.5 (3)		18.3 (3)	19.7 (4)	6.3		
7	9.0 (4)	3.4	12.4 (5)	10.5 (5)		15.1 (5)		2.7	11.5 (5)	3.6
8	10.5 (4)	—1.0	9.5 (4)	12.0 (6)		15.2 (4)		5.7	10.4 (4)	4.8
9	13.0 (8)	0.4	13.4 (8)		13.0 (8)				12.0 (8)	
10	13.0 (4)	—0.5	12.5 (4)	14.5 (4)		15.0 (4)		2.5	14.0 (4)	
11			20.0 (4)	20.0 (4)		24.0 (3)	26.0 (3)	4.0	19.2 (4)	—4.8
Average		1.3 ± 0.57 *						2.9 ± 0.63 *		3.5 ± 0.9 *

* Standard error.

compared to that of the oestrogen treated spayed control animals can be obtained. Hense, the clitoris is at least as sensitive to androgen as the castrate's prostate is (Wijnans, 1953). Bruzzone and Lipschutz, found the clitoris growth by androgen in spayed guinea pigs to be counteracted by oestrogen. The difference between their results and ours may be due to the much higher doses used by these authors (approx. 12 μg of oestr. benz. and 110 μg of test. prop. per 200 g bodyweight), or possibly to the different animal species. Hall (1938), using large doses of test. pr. (0.15-1.5 mg daily), saw no antagonistic effect of oestrone, but this may well have been due to the small doses used (6 μg, thrice weekly).

Our results suggest that the amount of androgen circulating in the female rat is at most approx. 1/10 of that in the male: 4 μg daily caused enlargement of the clitoris above the normal weight, whereas we had to administer 40 μg daily (whether or not combined with 2 μg of oestr. benz.) to castrated male rats of comparable weight in order to obtain seminal vesicles of normal weight (unpublished data).

The fact that in all our experiments oestrogen was also administered forms no objection to this supposition since normally the clitoris of the adult female will regularly be exposed to small concentrations of oestrogen. We should be certain, however, that the oestrogen doses administered did not cause the oestrogen concentration in the body to deviate markedly from the normal level. As a matter of fact the dosage of oestrogen aimed at a subnormal uterus weight and a somewhat supranormal pituitary and adrenal weight (see introduction) in an attempt to normalize these with androgen. Considering that the clitoris was found enlarged in all series but one (even in series 5, where the uterus weight showed but a very slight increase (119-144 mg) with oestr. benz. (0.05 μg daily) when 4 μg daily of test. prop. was also injected), and that in none of the series with intermittent oestrone-administration the oestrus periods reached normal length, we fancy to be safe in assuming that our oestrogen dosage cannot have been much higher than the theoretical dosage scheme which would cause the normal level (and the normal fluctuations of this level) in the body.

For the rest, it could be shown in subsequent experiments that 4 μg of testosterone propionate daily increases the weight of the clitoris of the spayed female to approx. the same extent as the combined treatment with 4 μg test. prop. and 0.5 μg of oestr. benz. does.

As regards the spayed female rat we conclude that it seems cautious not to think in terms of substitutional or "physiological" doses of androgen when more than 3 μg of testosterone propionate daily is administered.

SUMMARY

Small doses of testosterone propionate, e. g. 4 μg daily, applied alone or simultaneously with small doses of oestrogen, were shown to enlarge the clitoris of the spayed adult female rat above its normal size. Any normal concentration of androgen in the female must therefore, be considered, to be very low.

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CONSERVATION OF GONADAL TISSUE FOR TRANSPLANTATION

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MUCH of the work of Professor Lipschutz has involved the transplantation of the gonads in experimental animals, and I want to refer to one important feature of these researches, namely the conservation of ovarian tissue of the guinea-pig *in vitro* for subsequent transplantation, and to describe modern developments in this direction. In carrying out these experiments Professor Lipschutz used heterosexual transplantation, the criterion that an active graft of ovarian tissue had been established being the appearance of hyperfeminisation of the castrated male. Using this technique he observed that any treatment which damaged the tissue decreased the percentage of "takes", prolonged the period of latency and shortened the life of the graft. In experiments on storage above zero and on partial dehydration, grafts were obtained from tissue which had been kept at 1° to 3°C. for up to 16 days, at room temperature for up to 11 days, or which had been dehydrated to 50 % of its original weight. No "takes" were obtained from tissue kept at less than 0°C. or which had been dehydrated to 40 % of its original weight (Lipschutz, 1928 a, b, c, d, 1929, 1930, 1932; Lipschutz and Uprus, 1927; Lipschutz and Vesnakov, 1927). The results on storage below 0°C., though not unexpected, were somewhat disappointing in showing that under the conditions used by Professor Lipschutz it was not possible to preserve viability at a temperature low enough to be likely to permit of prolonged storage.

Recently, developments of this line of work arose from the discovery by Polge, Smith and Parkes (1949) that glycerol has remarkable properties in protecting livings cells against the effects of low temperatures. Application of this observation to mammalian ovarian tissue soon followed (Smith and Parkes, 1951). Detailed examination of variables associated with the technique (Parkes and Smith, 1953), showed that

rat ovarian tissue cut into small pieces and soaked in 15 % glycerol-saline could be frozen slowly to —190°C. in liquid air, and thawed without decreasing the percentage of "takes" when the tissue was grafted back to the ovariectomised donor. The time taken for an active autograft to become established, as determined by the re-appearance of cornification of the vagina, was, however, increased to approximately two weeks compared with the latent interval of approximately one week observed after the similar grafting of unfrozen tissue. In long-term experiments the duration of freezing was prolonged for up to one year without decreasing the percentage of "takes" or further increasing the latent interval (Table I).

TABLE I

Viability of rat ovarian tissue treated with 15 % glycerol-saline and frozen to —190°C. for various periods. (Viability determined by autografting and examination of vaginal smears).

Duration of freezing of tissue *days*	N° of animals receiving implantations	N° of animals in which active grafts developed	Average time taken for reappearance of cycle *days*
Not frozen	14	14	6.9
1/24	5	5	15.4
9	5	5	14.4
83	5	5	18.8
117	8	8	15.1
235	9	9	16.0
367	12	12	16.3

The use of other media and freezing to —79°C. (solid CO_2) gave less good results, and the experiments in general provided abundant illustrations of Professor Lipschutz's principle that increasing damage to the tissue before grafting is associated with a decreasing percentage of "takes" and an increasing latent interval.

Up to this stage, experiments had all been carried out using ovarian tissue removed from the body immediately after death. In considering the practical possibilities of the work, however, it seemed desirable to find out to what extent similar results could be obtained with ovarian tissue removed from the body some considerable time after death. The results (Table II) showed clearly that stale tissue makes grafts less readily and less effectively than fresh tissue, and that the combination of hazzards involved in freezing and homografting stale tissue results in a very low porcentage of "takes" and a long latent interval. Nevertheless, it should be emphasised that active grafts can be obtained from ovarian tissue removed from the body two days after death, and then frozen to —79°C. and thawed before implantation into another individual.

In analogous experiments on the male, 7-day old rats were killed and kept at + 2°C. for 0, 1 or 2 days. The testes were then removed,

TABLE II

Effect of leaving rat ovarian tissue in the dead animal at +2°C. before grafting. Tissue soaked in 15 % glycerol-saline and grafted with or without freezing to —79°C.

Time body at +2°C. *days*	Treatment of tissue	Nº of animals receiving implantations	Nº of animals in which active grafts developed	Average time taken for reappearance of cycle *days*
0	Soaked only	10	8	7.5
0	Soaked and frozen to —79°C.	9	6	13.3
1	Soaked only	10	9	10.3
1	Soaked and frozen to —79°C.	10	4	17.8
2	Soaked only	10	7	12.1
2	Soaked and frozen to —79°C.	10	2	20.0

TABLE III

Effect of leaving rat testis tissue in the dead animal at +2°C. before grafting. Tissue soaked in 15 % glycerol-saline and grafted with or without freezing to —79°C.

Time body at +2°C. *days*	Treatment of tissue	Nº of animals receiving implantations	Nº of animals in which active grafts developed
0	Soaked only	8	7
0	Soaked and frozen to —79°C.	8	7
1	Soaked only	5	5
1	Soaked and frozen to —79°C.	5	4
2	Soaked only	5	4
2	Soaked and frozen to —79°C.	4	3

soaked in 15 % glycerol-saline and grafted after, or without, freezing to —79°C. The results outlined in Table III show that testis tissue obtained and treated in such a way, retained viability remarkably well when left in the donor for up to 2 days after death and when frozen and thawed. Consideration of the size of the seminal vesicles of the host, however, shows that whereas testis tissue obtained one day post mortem behaves a'most normally as a graft, tissue obtained 2 days post mortem has a much diminished effectiveness as a graft. By the same criterion freezing to —79°C. is also a noticeable hazzard for the tissue.

The use of glycerol-containing media to protect cells against the otherwise fatal effects of freezing and thawing has started a new phase in the conservation of endocrine and other tissue for transplantation. Problems such as the mechanism whereby glycerol has this effect (Love'ock, 1953), the factors involved in the stability of the tissue at low temperature, optimal rates of cooling and so on are all under active and fruitful investigation (Smith - in press). By contrast, so far as the behaviour of the tissue after grafting is concerned we have little to add to the principles enunciated so clearly by Professor Lipschutz many years ago in the course of this oustanding researches.

The operative procedures recorded in this paper were carried out by my assistants Miss Jean Masson and Miss Mafalda Mansi, to whom my best thanks are due.

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ADRENALS AND GONADOTROPHIN B

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LIPSCHUTZ investigations [1] on the intrasplenic ovarian graft in castrated guinea pigs have led to the production of granulosa cell tumors or of luteomas. These tumors are attributed to the hepatic inactivation of the hormones elaborated by the ovarian graft, thus releasing the physiological regulation of the pituitary with a subsequent increase in gonadotrophins A (FSH) and B (LH) [2].

A few years ago, when we initiated our experiments with intrasplenic adrenal grafts in adrenalectomized rats, we hoped for the production of a similar phenomenon, that is, a hormonal desequilibrium brought about by a modification of the physiological control which the corticoids exert on the elaboration of corticotrophin. Soon it became obvious that the results obtained could not be compared with those produced by an ovarian graft. Furthermore, adrenal grafts in the neck region instead of in the spleen led to essentially similar results so that all important corticoid inactivation by the liver could be discarded. Finally, the grafted tissue never acquired a tumorous aspect, although it grew appreciably [3 to 8].

The most interesting aspects of the adrenal graft in adrenalectomized rats, quite unsuspected at first, resided not in the graft itself, but in the gonads, leading to a very definite hormonal imbalance, undoubtedly created by the graft and presumably with gonadotrophin B (LH) playing a major role. In this paper we shall deal with the experimental proofs for a possible influence of the adrenal on the elaboration of gonadotrophin B.

METHOD

The auto adrenal graft consists of approximately half an adrenal gland introduced within the spleen or alongside the trachea, in a juxtathyroidal position, in animals simultaneously adrenalectomized. Intrasplenic adrenal grafts were carried out in 368 adult rats and juxtathyroidal grafts in 225. Of these 593 animals, 325 (55 %) survived, the mortality being higher with intrasplenic graft. A 1 % NaCl solution replaced the

drinking water for the first month only; from onwards the mortality was almost nil. The animals were sacrificed at varying intervals from 15 days to 24 months after the adrenal grafting.

RESULTS

Histologically, the adrenal tissue presented a strikingly similar picture, independently of the location of the graft, of the presence of mesenteric adhesions or of the sex of the animals. The graft grew gradually and its aspect could only be related to the time of evolution.

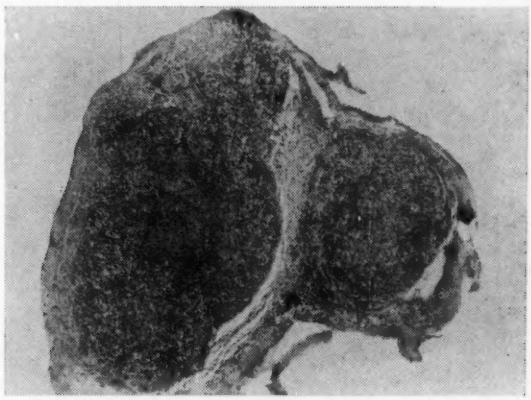

FIG. 1. — *Adrenal graft alongside the trachea.* Evolution, 1 year. Fascicular zone and glomerular zone on both sides of the capsule.

During the first 15 days, the grafted tissue goes to almost complete necrosis, after which it grows, gradually acquiring a more trophic appearance, with a cellular disposition characteristic of the fascicular zone, accompanied by intense capillary and arteriolar congestion. The capsule becomes denser. Subcapsular cells of the glomerular type are very scarce during the first 6 months after which they become more and more evident and more and more thophic in appearance, invading both sides of the capsule and often separated from the fasciculata by a well developed transition zone (Fig. 1). We have never been able to find any trace of reticular zone or of medulla.

As for the *pituitary*, there was no significant change in weight, but cytologically there were definite alterations. About 3 months after the adrenal grafting, there appeared in the anterior lobe groups of hypertrophied basophil cells, with homogeneous cytoplasm which later became finely vacuolated and were finally distended by a large vacuole. The

latter were not identical with castration cells, with which they alternated in castrated animals bearing adrenal grafts. The changes were more prominent in the presence of older grafts (more than 6 months) and more so in males than in females.

Since in previous experiments we had observed that the cytochemical distribution of ascorbic acid in the hypophysis can be used as an index of activity, we performed a number of determinations in grafted animals ([6]). Duting the first few months ascorbic acid was increased in the acidophils and in the chromophobes. In later grafts (more than 6 months) there was a shift towards the basophils with heavy concentration

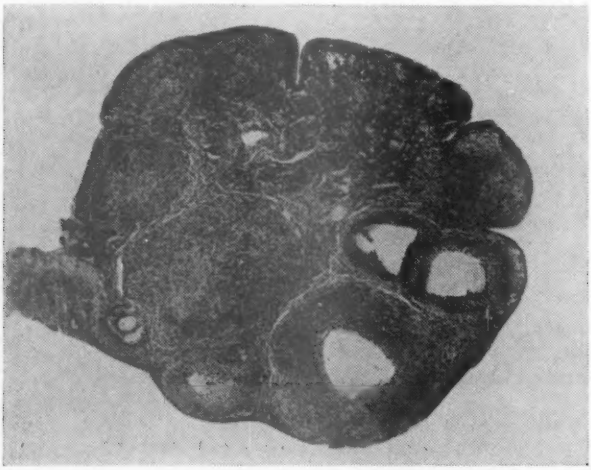

FIG. 2. — *Ovary of a rat bearing an adrenal graft alongside the trachea.* Evolution, 1 year. Numerous very trophic corpora lutea; follicle of De Graaf undergoing thecal luteinization.

in the hypertrophied finely vacuolated basophils, maintaining a certain increase in the chromophobes and disappearing from the acidophils.

The gonadal alterations were most striking. Even though in the presence of adrenal grafts of 3 months' evolution the changes were minimal, females bearing a graft of more than 6 months' evolution presented complete luteinization of the *ovary* (Fig. 2), with as many as 17 corpora lutea in one slide. Practically all primary and secondary follicles had ripened and no interstitial connective tissue persisted. In a few animals we have observed groups of very trophic interstitial cells. The corpora luea reached diameters around 500 microns and consisted of eosinophil cells with compact or finely vacuolated cytoplasm, that is, cells which seemed highly trophic; there were no corpora lutea undergoing involution. A few follicles of De Graff persisted, usually with luteinization of their theca. The uterus showed selective hyperplasia of the myometrium with practi-

cally no endometrial alterations. Daily vaginal smears had demonstrated that these animals were not in permanent estrus but in almost constant proestrus, with dyscariosis, a smear characteristic of pseudopregnancy.

The changes undergone by the *testes* were even more noterworthy. Again, in the presence of grafts of 3 months' evolution, the variations were slight and yet males bearing grafts of six months' evolution showed intense proliferation of Leydig cells, with definite hypertrophy and hyperplasia, at times forming voluminous nodules (Fig. 3). The cytoplasm of these cells colored intensely with eosin, was compact or very

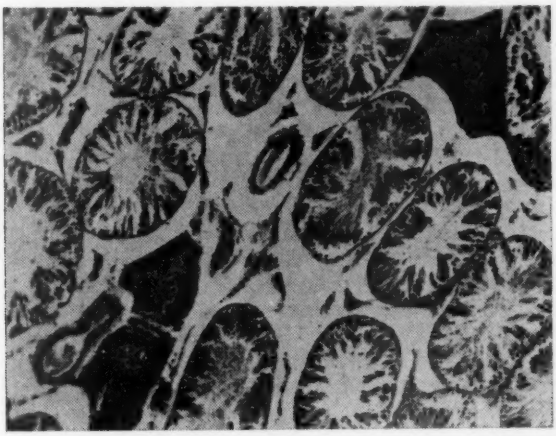

FIG. 3. — *Testis of a rat bearing an adrenal graft alongside the trachea.* Evolution, 1 year. Nodular hyperplasia of Leydig cells. Progressive detention of spermatogenesis.

finely vacuolated. The seminiferous tubules were separated by abundant edema, McManus positive and free of all phlogistic elements. These changes were accompanied by progressive detention of spermatogenesis which varied in intensity from one animal to the other; normal spermatogenesis with spermatozoa alternated with arrest at primary or secondary spermatocytes, alongside tubes devoid of all germinal elements and occupied solely by the Sertoli syncitium. The Sertoli cells remained normally trophic in appearance. The tunica propia and vascular walls showed no alteration from the normal.

DISCUSSION

The interpretation of the gonadal alterations brought about by the adrenal graft is most complex. It has often been emphasized that the pituitary-adrenal and the pituitary-gonadal interrelationships differ greatly. Bearing in mind the changes brought about by the ovarian graft, we expected the intrasplenic adrenal graft to undergo intense proliferation, due to an unchecked production of corticotrophin. This did not

happen. Even though the graft took readily and grew gradually, it never surpassed 35 mg in weight, which is approximately the normal adrenal weight. Its development remained the same whether it was located in the spleen of alongside the trachea. There seem no doubt however, that the production of corticotrophin is normal or increased, since the adrenal tissue has a very trophic appearance and since the eosinopenic response to adrenaline remains positive (4).

The profound gonadal alterations resulting in complete luteinization of the ovary and intense proliferation of the Leydig cells of the testes, leads one to think in a desequilibrium in the regulation of gonadotrophin B with an unchecked superproduction. The time factor is important here; we have seen that the ovary needs around 6 months to undergo complete luteinization. A comparable situation has been described by Biskind and Biskind (9) for the intrasplenic ovary; they suggest that all the follicles must develop before the luteoma can form. As for the persistence of corpora lutea, it would seem that constant hypophyseal stimulation is the important factor, implying a sustained elaboration of gonadotrophin B and probably of gonadotrophin C (luteotrophin) at the expense of gonadotrophin A. The testicular alterations could be explained by the same mechanism, the intense proliferation of the Leydig cells reminding us of the interstitial cell tumours of the testes which developed in some strains of mice when treated with estrogens. The latter have been assumed to arise in an environment of increased or continuous stimulation of endogenous gonadotrophin B (ICSH) (10).

The pituitary of these grafted animals shows no change in weight. Cytologically, the early changes are those characteristic of adrenalectomy, that is activation of acidophils; but in the presence of adrenal grafts of 6 months' evolution the basophils are affected selectively with hypertrophy and vacuolization. These cells differ from the castration cells with which they alternate in castrated and grafted animals. According to Pearse (11) an increased production of gonadotrophin B could be reflected in the basophils.

As for the grafted tissue itself, two facts are worth emphasis; a) the lack of any reticular zone, and b) the development of a glomerular zone which becomes very trophic in grafts of more than 6 months' evolution.

Thus we assume that the hormonal imbalance brought about by an adrenal graft in adrenalectomized animals resides in a superproduction of gonadotrophin B and not of corticortrophin. Two hypothesis are presented in an attempt to explain how the adrenal can affect the production of gonadotrophin B.

The first one would be to admit that the reticular zone of the adrenal is a physiological regulation of the secretion of gonadotrophin B, so that the complete lack of this zone would imply an unchecked production of this hormone. The time factor, the same as for the intrasplenic ovaries, would be necessary in order to abolish the elaboration of gonadotrophin A, once all the follicles have developed.

The second hypothesis could be based on the development of the glomerular zone of the adrenal graft which proliferates intensely in grafts of 6 months' evolution at the time when the gonadal alterations become

manifest. This zone normally produces corticoids of the type of desoxycorticosterone which have a definite luteoid effect. It is also the site of nodular hyperplasias observed by Houssay et al ([12]) in castrated rats.

We have a number of experiments underway in an attempt to prove one or the other of these hypotheses.

SUMMARY

An adrenal graft located in the spleen or alongside the trachea of adrenalectomized rats brings about a definite hormonal desequilibrium which resides not so much in the graft itself as in the gonads. The ovaries present a state of complete luteinization. The testes show frank hypertrophy and hyperplasia of the Leydig cells with progressive detention of spermatogenesis. In the hypophysis there is hypertrophy and subsequent vacuolization of basophil cells which are not identical with the castration cells. These changes become evident only when the graft has been growing for at least 6 months.

The adrenal graft whatever its localization grows gradually without ever surpassing the normal adrenal weight. It consists of an active fasciculata and few glomerular cells until around 6 months' evolution when a trophic glomerular zone proliferates, often on both sides of the capsule. The reticular zona is never present.

Two hypotheses are presented in an attempt to explain the mechanism behind this complex adreno-gonadal interrelationship. There seems to be a very warked increase in the elaboration of gonadotrophin B (LH or ICSH) which would depend on (*a*) the lack of a reticular zone, releasing a possible physiological regulation of gonadotrophin B, or (*b*) the hyperplasia of the glomerular zone with increased production of corticoids of the type of desoxycorticosterone.

RESUMEN

El injerto de suprarrenal en el bazo o en el cuello de ratas adrenoprivas provoca un desequilibrio hormonal manifiesto, no tanto en el tejido injertado sino en las gonadas. Los ovarios muestran luteinización completa. Los testículos presentan franca hipertrofia e hiperplasia de las células de Leydig con detención progresiva de la espermatogénesis. En la hipófisis se observa hipertrofia y vacuolización de las células basófilas. Estos cambios se esbozan a los 3 meses de evolución del injerto y se tornan francos a partir de los 6 meses. El injerto crece gradualmente sin adquirir en general un tamaño mayor del de una glándula normal, llamando la atención el desarrollo de una zona glomerular muy trófica recién a los 6 meses de evolución. La zona reticular falta por completo.

Se presentan 2 hipótesis para tratar de explicar el mecanismo responsable de estos cambios gonadales tan sugestivos de un gran aumento de secreción de gonadotrofina B (hormona luteinizante), que serían (a) la falta de zona reticular, suprimiendo un posible freno fisiológico de la gonadotrofina B, o (b) la hiperplasia de la zona glomerular con aumentada producción de corticoides del tipo de la desoxicorticosterona.

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COMENTARIOS SOBRE EL USO TERAPÉUTICO DE ESTEROIDES PARAHORMONALES

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EN 1942, Lipschutz formuló el concepto de la autodefensa tumoral basada en el mantenimiento de la homeostasis de esteroides. La aplicación de esta doctrina a la terapéutica paliativa ha dado resultados satisfactorios en muchos aspectos, y desde el punto de vista de la patología indica que la autonomía de las células neoplásicas es sólo relativa. La definición de tumor como masa de tejido en crecimiento desordenado debe revisarse para ajustarla, al menos en algunos rasos, a una manifestación compleja de trastornos de la homeostasis; y como siempre en la quimiostasis, han de considerarse los factores metabólicos y en particular, la inactividad o detoxicación de esteroides en el hígado. Lipschutz dedica un capítulo entero de su libro ([3]) a este problema, de muy amplios alcances. Como es bien sabido, los estudios de Lipschutz se han realizado en varias especies, especialmente en el sexo femenino, demostrando la aparición de tumores por relajación del control que el ovario ejerce normalmente sobre la hipófisis: como consecuencia de la fragmentación ovárica o castración subtotal se produce una desviación de la función gonadotrófica de aquella. La influencia ovárica sobre la hipófisis depende del ritmo en la producción, liberación y metabolismo de los esteroides.

La aplicación terapéutica de los estudios sobre tumorigénesis y autodefensa, se ha extendido a los dos sexos. Huggins, en 1938, señaló el efecto benefactor de la orquectomía y de la administración de estrógenos sobre el cáncer de próstata; y la administración de andrógenos y la castración se han utilizado en el tratamiento del cáncer uterino o mamario. "La finalidad del tratamiento del cáncer mediante hormonas sexuales es el aumento de la resistencia del paciente frente a la enfermedad" ([2]). O como diría Lipschutz, estímulo de la autodefensa antitumoral, más acción anabólica. En efecto, la retención de nitrógeno y mejoría del estado general, no pueden descartarse completamente, pero es probable que el efecto terapéutico se deba en su mayor parte a la neutralización de la acción tumorígena ejercida por el desequilibrio hormonal. En esta esquematización no debe olvidarse, sin embargo, que "la interpretración simple de los mecanismos anti-androgénicos en la evolución de los tumores,

aceptada hasta ahora, ha sido muy discutida recientemente" (1). Así, por ejemplo, los esteroides capsulares son también antitumorígenos potentes, en condiciones experimentales, y a la vez la actividad córtico-adrenal modifica la sensibilidad a la estimulación estrogénica (5). La limitación más importante para el uso terapéutico resulta de la aparición de caracteres de virilización en la mujer y de feminización en el hombre. Por esto, el paso siguiente ha sido el intento de empleo de esteroides artificiales con acción antogónica a las hormonas sexuales y sin efectos virilizantes o feminizantes desagradables.

Se pasa, pues, gradualmente, de la endocrinología a la farmacodinamia, y se sigue una vez más, el ciclo normal en la historia de la farmacología con los períodos sucesivos de identificación, extracción, síntesis y modificación estructural. Por observaciones en campos muy diversos, sabemos que pequeños cambios en la fórmula de cuerpos farmacodinámicamente activos modifican algunas de sus propiedades. En este caso, como en otros, los primeros términos son hormonas naturales sucesivamente identificadas, aisladas y sintetizadas, y los términos finales son creaciones sintéticas.

Entre los esteroides parahormonales, el más ampliamente explorado es el metilandrostenediol, que a pesar de ser muy similar a la testosterona ejerce efectos androgénicos muy mitigados y su administración a dosis terapéuticas prácticamente no produce alteraciones sexuales en la mujer. Causa retención de nitrógeno y contrarresta el efecto catabólico de la ACTH sobre las proteínas.

Se están explorando en la actualidad otros esteroides sintéticos con modificaciones diversas en la estructura molecular, que presentan actividad antiestrogénica sin efectos androgénicos, con la esperanza de poder emplearlos en los casos en que sea necesaria la acción antagonista. Varios de los cuerpos en exploración experimental interrumpen el estro en las ratas. Menos avanzada está la exploración de modificaciones moleculares de los estrógenos o de esteroides similares con acción antitestosterona, pero sin propiedades feminizantes.

Esta evolución encaja muy exactamente con las ideas de Lipschutz, quien comenta en su discurso de ingreso a la Academia (4) que su primera conferencia en Chile versó sobre la autorregulación orgánica (1926). que esta doctrina como la de autodefensa antitumoral, son aspectos de la medicina hipocrática, y termina diciendo: "Hipócrates sí, pero también Ehrlich". O en otras términos, corrección homeostática, pero también quimioterapia. Y Huggins escribe: *"Como agentes quimioterápicos*, las hormonas sexuales tienen grandes ventajas debido a su escasa toxicidad" (2).

Pero Lipschutz, después de una vida dedicada a la investigación fisiológica, no se contenta con esta afirmación superficial y señala con claridad que "el problema fundamental de la terapéutica con estrógenos no reside en buscar un compuesto menos tóxico o menos tumorígeno, sino en establecer las condiciones de tiempo de administración para que garanticen la acción discontinua del estrógeno" (3). Establece una diferencia clara entre uso de estrógenos, que deben administrarse de manera discontinua, para imitar el ritmo sexual, y tratamiento con otros esteroides, en que la continuidad de la acción es esencial. A pesar de su resistencia, bien justi-

ficada, a la adaptación inmediata de hechos experimentales complejos, es evidente la implicación de formas y pautas de medicación dúctiles, basadas en observaciones fisiológicas y farmacodinámicas, y muy diferentes de las viejas ordenaciones empíricas, siempre rígidas y rutinarias.

El sentido de la obra de Lipschutz, así considerada, es un lenitivo para quienes nos hemos desviado de camino, pues indica que nuestra ruta actual, aunque diferente en muchos aspectos, no es muy distante de la pretérita, con tantos pasos dados en su buena compañía.

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CUATRO ETAPAS EN EL CONOCIMIENTO DEL SISTEMA NEUROVEGETATIVO

AUGUSTO PI SUÑER

LA ANATOMÍA

GALENO (II A. D.) describe un "sexto" par craneal que corre por delante de las costillas, recibe fibras de las porciones torácica y lumbar de la médula y se distribuye por las vísceras abdominales. No distingue Galeno la cadena simpática del pneumogástrico. En igual error inciden los anatómicos del Renacimiento. Etienne (1545), Eustachio (1552-63), Vesalio (1555) dibujan la cadena simpática paravertebral y el vago, mas consideran también que tienen origen bulbar común. Será sólo en 1727, cuando Pourfour du Petit reconocerá que la cadena no es nervio craneal.

Por otra parte, Willis (1664), Vieussens (1710), Johnstone (1764), muestran la profusión de ganglios existentes en la cadena y nervios que de ella proceden y la presencia de plexos gangliformes en las lejanas ramificaciones simpáticas. Estos descubrimientos anatómicos conducen a suponer que ganglios y plexos tengan significación fisiológica. Willis (1664) había manifestado que en el animal se dan movimientos *involuntarios*, atribuibles a la intervención del simpático y distintos de los *volitivos*. Winslow (1732) propone la denominación de *simpático* para designar el sistema. Nombre que acepta Haller (1762) porque considera que, por dicho sistema, se establecerían, entre los órganos, las *simpatías:* entre la cabeza y el vientre, entre el espíritu y las funciones orgánicas.

Pourfour du Petit (1727) comprueba el origen dorso-lumbar de las fibras que van al simpático catenario, y describe particularmente el simpático cervical; después de que Haller (1743) hubiese estudiado los ramos comunicantes entre la médula y el correspondiente ganglio simpático de la cadena. Años más tarde, J. Müller (1834) y Valentin (1836) ven que los ramos son dos para cada ganglio: uno *blanco* —mielinizado— y otro *gris* —amielínico.

Boerhaave (1690) había antes afirmado que las funciones nerviosas son dobles —"homo simplex in vitalitate, duplex in humanitate"— fun-

ciones vitales y humanas; esto es vegetativas y racionales, involuntarias y voluntarias. Whytt (1751) confirma la tesis de Willis y de Boerhaave. Los movimientos voluntarios se hallan bajo la dependencia de la inervación central y los involuntarios, viscerales, de la inervación simpática. Tiempo después, Bichat (1800-01) precisará definitivamente la distinción entre las funciones nerviosas centrales y funciones simpáticas: sistema nervioso de la vida de relación o *animal*, y otro de la vida orgánica o *vegetativa*.

En el siglo XIX comienza la exploración microscópica del simpático. Ehrenberg (1833) descubre los somas celulares nerviosos existentes en los ganglios. Remak (1838) observa que las fibras que proceden de las células ganglionares son amielínicas y, más tarde (1854), explica, el mismo autor, la estructura de los ganglios, de las conexiones de las células que en los mismos se encuentran y las propiedades de las fibras del sistema. Meissner (1857), Auerbach (1864) describen respectivamente los plexos submucoso y mientérico intestinales. Experimentos de degeneración nerviosa y observaciones embriológicas de Balfour (1877-81) y Onodi (1885), exactas investigaciones histológicas de Cajal (1891-93), de Kölliker (1894) van facilitando que se incluya en un esquema la disposición del neurovegetativo. Gaskell (1896-1916), separa el simpático, cuyas fibras emergen de la región tóraco-lumbar de la médula, del parasimpático, de origen bulbar y sacro. Prueba, además, que los ganglios son lugar de articulación entre fibras de origen neuroaxil —mielínicas— *preganglionicas*, y las células del ganglio, de las que parten las fibras *postganglionicas* —amielínicas— las cuales, saliendo del ganglio, van a distribuirse por los órganos. Simpático y parasimpático forman el sistema que Gaskell (1916) llama *involuntario;* que Langley (1921) ulteriormente denominará *autónomo;* y al que más tarde se le ha devuelto el nombre clásico de sistema nervioso vegetativo o *neurovegetativo*.

Fisiología y Clínica

En 1727, Pourfour du Petit secciona el simpático cervical en animales y comprueba la consiguiente vasodilatación paralítica del lado correspondiente de la cara. Numerosos investigadores confirman, en distintas fechas, el fenómeno. Cl. Bernard (1851-56) estudia el caso y ello le conduce a investigaciones muy importantes sobre fisiología del simpático: los efectos de la sección o de la excitación de distintas regiones del sistema, diferenciando la influencia funcional del simpático de la del vago y otros nervios parasimpáticos.

Gaskell (1886-1916) atribuye influencias antagonistas, en la inervación visceral, a simpático y parasimpático, actuando sobre la fibra lisa, las glándulas y el trofismo. El simpático sería, en general, estimulante, desasimilativo; y el parasimpático inhibidor, asimilativo. Langley, por una labor dilatada que empieza en 1891, consigue resultados valiosos. Mediante la intoxicación por la nicotina, bloquea al ganglio simpático y, de este modo, la corriente que viene de los centros para estimular la célula ganglionar, no pasa a la fibra postgangliónica. Así va explorando Langley la actividad de distintas regiones del sistema. En cuyo sistema in-

plexo el simpático de una parte; y de la otra el vago y otros nervios craneales eferentes y nervios pelvianos, que constituyen los conductores del parasimpático. Reconoce, además, la existencia del *sistema entérico*, plexiforme ganglionar, en las vísceras, encargado de decidir localmente de las funciones de las mismas.

El hecho de que, realmente, los efectos de la excitación de los nervios pertenecientes al simpático suelan ser opuestos a los que se consiguen al excitar los nervios parasimpáticos, dió lugar a deducciones clínicas apresuradas. Eppinger y Hess (1910) publican "Die Vagotonie"; y antes Rosenbach (1897) había definido la *neurosis del vago*. Aumento en el tono o en la excitabilidad del vago provocará un estado *vagotónico;* lo contrario se traducirá en *hipovagotonía*. Habrá, contrariamente, si es el simpático el afectado, posibilidad de *simpaticotonía* o de *hiposimpaticotonía*. Y se hablará de *distonías* y de *anfotonías*. Creándose, así, un sistema médico que, como todos los sistemas, habría de lograr general aceptación. "La doctrina de la vagotonía y la simpaticotonía —escribe Bergmann (1930)— se extendió rápidamente en la práctica, dando lugar a que, con enorme ligereza y sin crítica alguna, llegara a sustituirse con una palabra un diagnóstico justificado". Durante unos años toda la patología del neurovegetativo se resumió en tales términos. Suponíanse alteraciones de unos nervios simplemente o, cuando más, de los núcleos de origen de dichos nervios.

Funciones y disfunciones centrales

Se conocía, no obstante, desde largo tiempo la influencia de centros bulbares y medulares sobre diferentes funciones vegetativas. Legallois (1812), los hermanos Weber (1845), Bezold (1862) habían probado la intervención de algunos de aquellos centros en la actividad cardiaca; Legallois (1812), Flourens (1824) en la respiración; Cl. Bernard (1856) en la función glucogénica del hígado, etc., etc.

En 1897, Paton observa que la picadura del diencéfalo es causa de de hipertermia y de hiperglucemia simultáneamente. Dejerine y Roussy (1906) y Kasplus y Kreidl, por una dilatada serie de trabajos (1909-37), y Camus y Roussy (1913) y Roussy con diferentes colaboradores (1907-1937), Phillip Bard (1928-34), etc., prueban la tan importante intervención del hipotálamo, y también del tálamo, en el gobierno de las funciones vegetativas. Bien pronto se demuestra la parte que toman asimismo en ello ciertas regiones de la corteza cerebral.

La observación clínica permite comprobar inmediatamente que lesiones hipotalámicas dan paso a enfermedades del neurovegetativo: la epilepsia vegetativa de Penfield (1929), los ataques parasimpáticos de Cushing (1932), ulceraciones digestivas (Cushing, 1932), diabetes insípida (Kernohan, Learmonth y Doyle, 1932), etc. De antes se conocía la participación del diencéfalo en la patología del sueño (Mauthner, 1890; von Ecónomo, 1917-31). Una muy larga serie de investigaciones en el hombre, con necropsia, y de experimentos sobre animales vienen a comprobar lo mucho que cuenta en la fisiología y en la patología neurovegetativas el estado de los centros a diferentes niveles neurales. Esto es,

que no todo, en las enfermedades del vegetativo, son alteraciones funcionales del simpático o del vago.

La Química

Lewandowski (1900) y Langley (1902), observan que la inyección de adrenalina da lugar a iguales efectos que la excitación simpática; y supone, en consecuencia, Elliot (1905) que, en la excitación del simpático, sea, dicha adrenalina, el estimulante químico obrando sobre el órgano efector; cada vez que el influjo nervioso llegue a las terminaciones en dicho órgano. Dixon (1904-07), Weiland (1912), Demoor (1930), Loewi (1914-22), prueban que, igualmente, al excitar los nervios del parasimpático —el vago especialmente— se acumulan, en los tejidos inervados, substancias capaces de por sí de provocar efectos idénticos a los de aquella excitación. Dale (1914) y Dixon (1914), piensan que tal substancia sea la acetilcolina, idea que ha sido después totalmente confirmada.

La excitación de nervios simpáticos y parasimpáticos libera respectivamente, en las terminaciones de los mismos o en su proximidad, adrenalina —o noradrenalina— y acetilcolina; *substancias intermedias*, según las denominara Langley (1921). Es así como Dale (1933) propuso llamar terminaciones *adrenérgicas* a las del simpático y *colinérgicas* a las parasimpáticas.

Se ha visto pronto que la acetilcolina interviene también en el proceso de transmisión del estímulo nervioso en la sinapsis ganglionar vegetativa (Dale, 1933; Kibjalow, 1933; Chang y Gaddum, 1933), en las sinapsis centrales del neuroeje (Sherrington, 1925; Loewi, 1936, y Chang, 1938), y en la placa motriz del músculo estriado (Bremer y Rijlant, 1924; Feldberg, 1933; Feldberg y Dale, 1934).

Y no hay que considerar únicamente la acetilcolina y la adrenalina como mediadores en los procesos de inervación neurovegetativa. Otros productos endocrinos o del metabolismo manifiestan propiedades referibles a las de aquellas substancias. Dale y Dixon (1909) habían visto la acción simpaticomimética de la tiramina. Mientras que Lewis (1927), Ungar (1936), muestran que la histamina; Liechti y von Muralt (1931), Minz (1938), que la tiamina; y Drury y Szent Giorgy (1936), que el ácido adenílico y su nucleósido, la adenosina, son parasimpaticomiméticos. Y otras muchas más substancias, elaboradas por el organismo, parecen intervenir en la transmisión de estímulos vegetativos. Demoor (1938), propone por eso la denominación de *substancias activas;* numerosas, más o menos complejas en su composición y estructura —desde electrolitos hasta moléculas gigantes— cooperando en la regulación neurovegetativa, en el funcionamiento de los órganos.

Por otro lado, la íntima relación existente entre la actividad del neurovegetativo y la producción endocrina y efectos hormónicos —lo cual hizo que Pende (1922), creyese en la realidad de un *sistema neuroendocrino* y Kraus (1929) de un *sistema vegetativo*, químico y nervioso a la vez— enseña la estrecha conexión de los dos grandes mecanismos de control fisiológico. Desde la colusión adrenalino-simpática (Fusari, 1893; Kohn, 1906; Da Costa, 1917), hasta la compleja sinergia anótomo-fun-

cional hipófiso-hipotalámica (Scharrer y Scharrer, 1937-40) —por lo cual Roussy y Mosinger (1933) hablaron de *hipofisopexia, coloidopexia neuronal*, de *neurocrinia*, de *neuricrinia;* es decir, de fijación por la hipófisis de productos segregados por células nerviosas diencefálicas; y, viceversa, de efectos sobre tales centros del diencéfalo provocados por hormonas procedentes de la hipófisis— vése claramente la asociación funcional de agentes químicos y neurovegetativo. La resonancia de la actividad solidaria del complejo hipotálamo-hipófisis sobre la función de diferentes glándulas endocrinas, particularmente tiroides y suprarrenal —con su médula y sobre todo su corteza, de tan grande influencia sobre gónadas y otras glándulas— nos muestra cómo es de complicado el ajuste neuroquímico, presidiendo la vida de los órganos! Se dan constantemente cruces, paralelismos, adiciones o inhibiciones entre los grandes procesos de coordinación vital: químicos y nerviosos.

Ello extiende enormemente el concepto de regulación vegetativa. Desde los centros del neuroeje hasta la periferia —es decir, el órgano, el tejido, la célula— la atmósfera química y físico-química que rodea la tal célula, y en la cual ella vive, decide, conjuntamente con el neurovegetativo, del funcionamiento de la misma. Llegamos aquí, a las raíces moleculares, micelares, de la vida. Y esta es la etapa —en la evolución de nuestros conocimientos— que vivimos hoy. Bien distinta, y más amplia, más comprensiva en sus conceptos, que aquellas que la precedieron.

THE EFFECTS OF PROGESTERONE AND RELATED COMPOUNDS ON OVULATION AND EARLY DEVELOPMENT IN THE RABBIT *

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THAT PROGESTERONE is an effective inhibitor of ovulation was suggested by the difficulty of inducing ovulation in animals in which the ovaries contain active corpora lutea (Parkes, 1929). Direct demonstration of the ovulation-inhibiting effect in the rabbit was made by Makepeace *et al.* (1937), in the rat by Astwood and Fevold (1939), and in the sheep by Dutt and Casida (1948). Since progesterone also appears to inhibit fertilization in the rabbit (Boyarsky *et al.* 1947), we became interested in the further study of these phenomena and particularly if the ovulation-inhibiting effect and/or the fertilization-inhibition might be differentially affected by different substances. The mode of administration we have employed has failed to give any clear indication of an effect upon fertilization of the various compounds employed, but our data on ovulation inhibition are fairly clear cut, and seem worth recording.

METHODS AND MATERIALS

Mature rabbit does in estrus and of miscellaneous stocks were employed. The steroids employed in these studies were, with a few exceptions to be noted, administered in a single dose, and at appropriate intervals thereafter the does were mated to mature males. Twenty-four hours following the mating a laparotomy was performed and the occurrence of ovulation determined by the presence or absence of corpora lutea in the ovaries. If corpora lutea were found one fallopian tube was excised (and on ocasion the uterus on the same side), the incisions were sewn up, and the animal kept for further observation. The excised fallopian tube was flushed for ova which were then examined to determine if fertilization had taken place. Fertilized ova taken at this time are readily recognizable by the presence of cleavage and of sperm in the

* The investigations described in the paper were aided by grants from the Planned Parenthood Federation of America, Inc., the Ciba Co., Inc., and the Chemical Specialties Co., Inc.

TABLE I

Effects of a single subcutaneous injection of a macrocrystalline suspension of progesterone on the ovulation, transportation of spermatozoa and fertilization in the rabbit.

(The female rabbits were bred twice with fertile bucks at various days after injection, and examined 1 day after breeding).

Dose	Rabbit	Time of breeding: days after injection	Ovulation	Fertilization	Nº of sperm in the tubes	Nº of sperm in the uteri
10 mg	1	1	No	—	24 880	653 600
	11	1	No	—	0	1 976 700
	3	4	No	—	0	0
	4	8	Yes	4 of 16 ova	0	133 950
	13	8	No	—	2 667	1 836 00
	6	11	No	—	0	0
	9	14	Yes	7 of 9 ova	5 976	394 200
	14	14	Yes	9 of 9 ova	2 750	177 000
30 mg	2	1	No	—	4 140	8 300
	12	1	No	—	31 750	2 620 500
	8	4	No	—	0	0
	5	8	No	—	0	6 000
	7	11	No	—	0	142 240
	10	14	No	—	3 726	1 224 850
	15	18	No	—	0	12 834
	16	24	No	—	—	—

zona pellucida and/or the perivitteline space. In certain instances counts of sperm in the flushings from the tube and the uterus were also made. Nine to eleven days later, a second laparotomy was performed and the uterus on the unoperated side examined for implantations.

Results

Before proceeding to a detailed account of our various experiments, we should state at once that in practically every instance in which ovulation occurred fertilization was observed if mating with fertile males was practised and implantation of fertilized eggs also took place. The ensuing

TABLE II

The effects on ovulation in rabbits of the intravaginal deposition of progesterone, 17α-hydroxyprogesterone and allopregnene-3β-ol-20-one. (The female rabbits were bred twice with bucks at various times after the deposition and examined 1 day after breeding).

Substance	Dosage mg	Nº rabbits	Time of breeding: hours after deposition	Nº ovulating
Progesterone	30	1	24	0
	10	1	24	0
	1	2	24	0
	1	2	5	0
	1	2	2	2
	0.5	1	24	1
	0.1	1	24	1
17α-Hydroxy-progesterone	2	4	24	0
	1	1	24	1
	0.5	1	24	1
Allopregnanolone	5	2	24	1 (2)
	5 (1)	2	24	2

(1) Subcutaneous injection.
(2) Fertilization and implantation occurred.

account is therefore concerned with the influence of various substances on ovulation.

Our initial observations were concerned with the effects of progesterone injected subcutaneously as a macrocrystalline suspension. The data on the effects of 10 mg and 30 mg doses are presented in Table I. It is clear that the higher dosage effectively blocked the ovulation that normally follows copulation. In one instance ovulation occurred et eight days, and in two instances at fourteen days following the lower dose, suggesting somewhat reduced effectiveness of the lower dosage. Although great irregularities in sperm count were had, there is no obvious difference between the two dosages in relation to the frequency of appearance or number of sperm in either the fallopian tubes or the uterus.

In Table II we present data on the effects of the intravaginal deposition of various amounts of progesterone, 17-α-hydroxyprogesterone and allopregnanolone on ovulation. The data demonstrate: (a) That if mating is practised in less than five hours after the intravaginal administration of 1 mg of progesterone ovulation will occur; (b) that if mating

takes place at 24 hours after the deposition of 1 mg of progesterone the block to ovulation is established as well at this dosage as at 10 and 30 mg dosages; (c) that intravaginal dosages of 0.5 and 0.1 mg are not effective at 24 hours following administration; (d) that 2 mg intravaginal dosages of 17α-hydroxyprogesterone are effectively ovulation-inhibiting, but that dosages of 1 mg or less are probably ineffective; and (e) that allopregnanolone is probably not effective as an inhibitor of ovulation by either the intravaginal or subcutaneous route at a 5 mg dose level.

TABLE III

Effect of oral administration of ethinyl testosterone on ovulation, fertilization and implantation in the rabbit. (The animals were bred twice 1 day after feeding and examined 1 day and 6-7 days after breeding).

Dosage	Nº Rabbits	Nº Ovulating	Fertilization	Implantation
10 mg in a single feeding	8	1	? (1)	—
5 mg in a single feeding	13	8	Yes (2)	Yes (2)
2 mg in a single feeding	6	4	Yes (2)	Yes (2)
1.20 mg daily for 5 days	4	2	? (1)	—
1 mg daily for 5 days	4	2	?	No
0.75 mg daily for 5 days	2	2	?	—

(1) Bred to a sterile male.

(2) In females mated to fertile males.

Ethinyl testosterone (an oral progestin) was administered by mouth in various courses as indicated in Table III. It will be noted: (a) That seven out of eight rabbits given 10 mg in a single dose by mouth failed to ovulate, whereas five out of thirteen failed at the 5 mg and two out of six at the 2 mg levels; (b) that 1.25 mg and 1.0 mg fed daily for five days were partially effective; and (c) that dubiously irregular effects on fertilization and implantation occurred.

In Table IV we present data on the effects of the administration of 17-methyl progesterone in various experiments. The results suggest the relative ineffectiveness of the oral route, but an effectiveness by subcutaneous or intravaginal injection comparable to that obtained with progesterone (*cf.* Table II) and with a duration of the effect at 5 mg comparable to that seen with progesterone (*cf.* Table I).

Intravenously into three rabbits one day after the subcutaneous injection of 17-methyl progesterone, we injected a pituitary extract at a

TABLE IV

The effects of 17-methyl progesterone (dissolved in propylene glycol) on ovulation in the rabbit. Each rabbit was given the preparation in a single dose, and the determination of ovulation made 24 hours after mating.

Dosage mg	Nº of rabbits	Route of administration	Time of mating after administration, days	Nº ovulating
5	3	Subcutaneous	1	0
10	4	„	1	0
5	2	Intravaginal	1	0
10	2	Oral	1	2
50	2	„	1	1
5	2	Subcutaneous	2	0
5	3	„	6	0
5	2	„	7	1
5	2	„	8	2

dosage level which was invariably ovulating in normal estrus females. Two of the three had ovulated when examined 24 hours later, suggesting that the methyl progesterone effect may involve a block to the release of pituitary gonadotrophin.

Using a standard tehnique of subcutaneous injection in 10 mg dose followed by mating in 24 hours, we have examined a series of pregnane and allopregnane derivatives as possible ovulation inhibitors. The data are presented in Table V. With the possible exception of pregnane-3,20 dione, no suggestion of ovulation inhibition at this dosage is had for any of the compounds tested.

Discussion

The effectiveness of progesterone as an inhibitor of ovulation is reaffirmed by our data. Furthermore, certain active progestins, namely ethinyl testosterone and 17-methyl progesterone are also effective. Since 17-hydroxyprogesterone is also effective, it is suggested that substitutions at carbon 17 in the progesterone molecule do not notably interfere with the ovulation-inhibiting capacity. It is a matter of great interest that 17-methyl progesterone is roughly twice as active as progesterone in standard assay (Heusser *et al.*, 1950) whereas 17α-hydroxyprogesterone is inactive as a progestin (Pfiffner and North, 1940). A dissociation of progestational and ovulation-inhibiting effects is suggested. On the other hand, the ineffectiveness of 11α-hydroxyprogesterone at a dose notably effective for progesterone and 17-methyl progesterone suggests that substitutions at carbon 11 may interfere with ovulation-inhibiting capacity. It is notable (Byrnes *et al.*, 1953), that 11α-hydroxyprogesterone has less than one-half the activity of progesterone as a progestin, but in the

TABLE V

The effects on ovulation of various pregnane and allopregnane derivatives. Each compound, dissolved in propylene glycol, was administered in a single 10 mg. dose. Mating was practised 24 hours later, and examination for ovulation at 24 hours following mating.

Compound	Nº of females tested	Nº ovulating
Allopregnane-17α-ol-3, 20-dione	3	3
Allopregnane-3β, 17α, 21-triol	4	4
Allopregnane-21-ol-3, 20-dione	3	3
Pregnane-3, 20-dione	3*	1
Pregnane-3α, 20α-diol	3	2
Pregnane-3α, 20β-diol	3	3
Pregnane-3β, 20β-diol	3	3
$\Delta^{4\ 16}$-Pregnadiene-3, 20-dione	3	3
11α-Hydroxyprogesterone	3	3

* 1 animal with small ovaries.

immature rat is only slightly less active as a pituitary gonadotrophin inhibitor. No reduction product of progesterone (with the possible exception of pregnane-3,20-dione) appears to have any significant ovulation-inhibiting capacity. Further studies of such compounds at varying dosages are, however, clearly necessary to rule out the possibility of any effect. We hope to report on such experiments as well as experiments with other types of progesterone relatives at a later date.

Some remark should be made about our failure to observe any effects on ovum fertilization. Boyarsky *et al.* (1947), found that pretreatment for ten days at 2 mg per day was necessary to block fertilization 95 %; the same dosage in a pretreatment for five days was completely ineffective. Also they had to ovulate the rabbits artificially with pituitary extract and artificially inseminate. It is clear that our experiments have been conducted under a different regime and that the fertilization-inhibiting treatment is inhibiting to ovulation induced by mating.

SUMMARY

The potency of progesterone as an inhibitor of ovulation in the rabbit has been reaffirmed. Its effectiveness when administered by subcutaneous or intravaginal routes is demonstrated. A single injection of 30 mg of progesterone may prevent ovulation for as long as 24 days.

Ethinyl testosterone administered orally is an effective inhibitor of ovulation in the rabbit, and the effect appears to be proportional to the dose over the range 2 to 10 mg in a single feeding. 17α-Hydroxyprogesterone administered intravaginally is an ovulation inhibitor. 17-Methyl progesterone is quite effective when administered intravaginally or subcutaneously, but is relatively ineffective when given by mouth. A series of ring-A reduced allo and normal reduction products of progesterone tested by subcutaneous injection at the 10 mg dose level appeared to be ineffective as ovulation inhibitors. 11α-Hydroxyprogesterone also is ineffective at this dosage.

Acknowledgements

We are indebted to Dr. I. V. Sollins of the Chemical Specialties Co. and to Dr. E. Oppenheimer of the Ciba Co. for supplies of compounds used in these studies. Miss Betty Hull offered valued technical assistence.

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CANCER RESEARCH AS VIEWED ALONG THE YEARS

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EVERY working scientist has two roles, those of doer and onlooker. The latter role is usually the more contenting to workers at cancer, —whose ordinary lot is to accomplish little, so obdurate is the problem. Looking along the years though, one can perceive that this is slowly giving way, now here, now there; things do get found out though one may not oneself find them. My aim now is to remark upon the various forces, observational, experimental, psychological, social, and accidental, which have determined the course of cancer research since it really began, some fifty years ago.

Not until nearly the turn of the century did pathologists become generally aware that the cancers of animals can be maintained in host after host. Then hope ran high that through scrutiny of the growths thus available the cause of the disease might be laid bare. Scientific personages, both great and small, made the attempt and funds were swiftly forthcoming for it; but all to little avail after ten feverish years. The cancer cell still held its secret close. Furthermore it was found to elicit no immunity worth practical note. Shrewd investigators betook themselves to more promising themes, public interest wandered away, and the defeatist aphorism gained currency that the cancer problem would be solved only with that of life itself.

From 1910 to 1915 cancer research lay in the doldrums. Young scientists with careers to make were actually warned off from it, and those few investigators who kept at it had either a compelling curiosity or endowed jobs. Yet glints of future discovery shone out during this period. The mice from a certain breeder were noted to have mammary cancers with singular frequency; and the failure of tumors after transplantation was traced to differences in their mongrel hosts. These facts presaged a genetics of cancer. Also tests showed that ovariectomy or often-repeated pregnancies in mice markedly affected the incidence of mamary cancer. Obviously hormones had a hand in its occurrence. But in that day neither the hormonal nor the genetic lead could be

followed for lack of knowledge and materials. Certain tumors of chickens were proved due to viruses; but this disclosure, which conflicted with the negative findings in mammalian growths, was dismissed forthwith as erroneous, and it remained in limbo for years after. A surgeon who had made a few human tumors recede, by producing high fever with pyrexial toxins, dinned into any listening ear the story of his success. But few were the ears that listened. Too many had been the vain efforts at cure. One must understand tumors first —so ran the thought of the day— and indeed new understanding had just previously led to many triumphs over disease.

In 1915 a wind of discovery swept in; cancers were induced at will by tarring the skin of rabbits and mice. No one had previously known more of how animal tumors come about than the physician of today knows of most human cancers when brought to him. Now at last their course could be followed from the first barely perceptible lump. Many workers undertook the task.

Yet all still went slowly. Injected tar is so ill-borne that few internal tumors can be got by its means; and though teeming with ingredients it often lacks the cancer-inducing principle. This was not isolated for more than a decade, and only then because several closely integrated scientists, each with a special knowledge or gift, gave themselves to the problem. The agent proved to be a complex hydrocarbon, and soon related substances were synthesized, some acting much more powerfully in the same peculiar way. Here at last, striking hard at the cancer problem was biochemistry! It had been deeply revealing some little while before when it disclosed that the glycolytic activities of tumors differ distinctively from those of normal tissues, a fact indicating some basic relationship amongst the neoplasms. Despite this, most workers with the agents inducing cancer still gave little attention to such other growths as they called forth. To obtain cancer was the engrossing wish. (The term *carcinogen*, still current, records this purblind view). Only gradually was it perceived that the agents inducing cancer elicit the whole range of the neoplasms, benign and malignant, — are *oncogens* in short; and with this realization came a greater one, that the tumors, however protean in their manifestations, have an underlying unity, that whatever the nature of the cell change responsible for the neoplastic state it is common to them all.

The intentness on cancer blurred the vision of workers in yet another direction. In their desire to obtain actively malignant growths like those of man, to validate their studies, they made nothing of the fact (though reporting it) that many of the carcinomas they induced vanished after a time. Only very recently have they learnt, from increasingly various instances, that a large proportion of the cells, perhaps most, which become neoplastic for one reason or another, require extraneous aid to form a tumor, and that the ravaging, independent entity, clinical cancer, is often an end product which has travelled a long, vicissitudinous way since its hesitant, benign beginning.

How often oncogens arise within the body is still an unanswered question. We now know however that hormones may so prepare the

ground for neoplastic change in mice and rats as to determine it, and that they actually urge on some of the worst human cancers and can be used to hold some back. Advances in biochemistry and its dependent science, endocrinology, have made this possible, if with no such end in view. The endocrinology of neoplasms has proved itself amongst the most rewarding themes of the day. Well do we know this who have followed with admiration the achievements of Alexander Lipschutz!

Genetics too has provided unforeseen enlightenment. Untangling the skein of heredity in mice by inbreeding (much as if human brothers and sisters had been mated successively since the Pharaohs), geneticists have uncovered the fact that pure strains of these animals differ markedly in tumor liabilities; and pathologists have now shown that the growths mots readily induced by the oncogens in this or that strain are those expressive of its natural liability. What can be the character of this? The answer has been provided in one instance by another, very startling discovery of the geneticists, but in only one as yet despite active seeking. They found, against all preconceptions, on cross-breeding mice of a strain highly liable to "spontaneous" mammary cancer with those of a strain almost free from it, that the tendency to the disease is passed on through the mother by way of her milk, and that the milk contains a virus which somehow causes cancer to occur in the nurslings receiving it, after they have matured, yet which is innocuous on all immediate tests. Prior to these revelations the geneticists had explained tumors as due to somatic mutations, and they still hold hard to the thought, like many other workers.

This peep-hole view of cancer research along the years, though narrow and cursory, shows how human beings strive to find out what they needs must know, when having almost no knowledge to go upon. It is indeed a type instance: persistent effort, ardent on occasion, many lifetimes of lost motion, much boggling, much blindness, much dogmatism, —Nature with sardonic humor showing the investigator again and again what a fool he is in his *a priori* pride—, and yet discovery made after discovery, not infrequently through accident and acumen working together.

Two great truths stand forth from out of these fifty years. One is that the pace of man's advance against the cancer problem has been that of the basic sciences. They can be counted upon to reveal in no long time, incidentally to their own development, the underlying character of neoplastic change; and more and more they are bringing aid to the tumor clinic. Time was when pathologists made the prime contribution to this by their correlation of the aspect of neoplasms with their behavior. Now one cannot say from which quarter in science the next boon will come. An anatomist, preparing himself all unaware by studying the cytology of the oestrous cycle in rats, has lately given the clinic a valuable method for learning the hidden presence of those many tumors that cast off cells into exudates, secretions, and excreta.

The second truth has only now struck home. It is this: when an unknown is lethal, killing myriads of people every year, one should not, cannot, wait to understand it or even, in striving against it, hold to the "scientific approach", limit one's effort to the likely. In fighting cancer we must

follow up each clue, however odd or seemingly irrational, search all about for weapons, converge on the dread thing from every point. Incredible though it seems, society has maintained until now a hands-folded belief that nothing can be done for cancer which isn't being done, —last remnant of the mediaeval attitude towards most fatal diseases. The mere commissioning of a few diligent, serious individuals to learn more about cancer has lulled the public conscience. But now, what with the making of the atomic bomb, men have abruptly perceived the potentialities of massed scientific effort. So today society demands as never before that the cancer problem be solved, and it pours out gold for the solving. The task is deeply different from that of the bomb; for it means coping with what is still inscrutable. But the antibiotics are doing now what rationalists had deemed impossible, and the knowledge already gained on the cancer problem gives ground for great hopes.

THE "CRITICAL PERIOD" IN THE DEVELOPMENT OF INFLAMMATION

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THE EARLIER work of this laboratory had shown that the typical response of the body to systemic stress is the "General Adaptation Syndrome" (G-A-S), which is characterized by manifold morphologic and functional changes occurring throughout the organism. Topical stress, on the other hand, elicits a "Local Adaptation Syndrome" (L-A-S), whose principal repercussions are confined to the immediate vicinity of the eliciting injury. The concept of the L-A-S has recently been outlined extensively in our contribution to the "Symposium on the Mechanism of Inflammation" (1). Its most salient features are: on the one hand, degeneration, atrophy and necrosis, on the other, inflammation, hypertrophy and hyperplasia. Its chronologic evolution is schematically outlined in the key-diagram of the above mentioned communication.

At first sight, there appears to be no striking similarity between the systemic and the local reaction-type. A patient, in traumatic shock, furnishes a characteristic example of the G-A-S and, in particular, of its earliest stage, the "shock phase" of the general alarm reaction. An abscess, formed around a splinter of wood, representes a typical example of the L-A-S and, in particular, of its "stage of resistance", during which the defensive inflammatory phenomena predominate. On the surface, these two instances of disease reveal no striking similarity and yet, more careful study shows them to be closely related.

The voluminous literature which deals with these basic patterns of reaction to what we call non-specific biologic "stress", has been surveyed elsewhere (2, 3, 4, 5). Let us re-state, however —for a better understanding of the problem which led us to perform the experiments reported here —that, among other things, the G-A-S and the L-A-S are thought to be interrelated because:

(1) both are *non-specific* reactions, comprising damage and defense;
(2) both are *triphasic*, with typical signs of "crossed resistance" (or, depending upon the stressors used, "crossed sensitization"), during the second stage;

(3) both are singularly *sensitive to the so-called "adaptive hormones"* (ACTH, STH, corticoids) ;

(4) they greatly *influence one another*, if the two reactions develop simultaneously in the same individual; that is, systemic stress markedly alters tissue-reactivity to local stress and *vice-versa*.

The object of this communication is to present additional data, demonstrating the fundamental similarity between the G-A-S and the L-A-S.

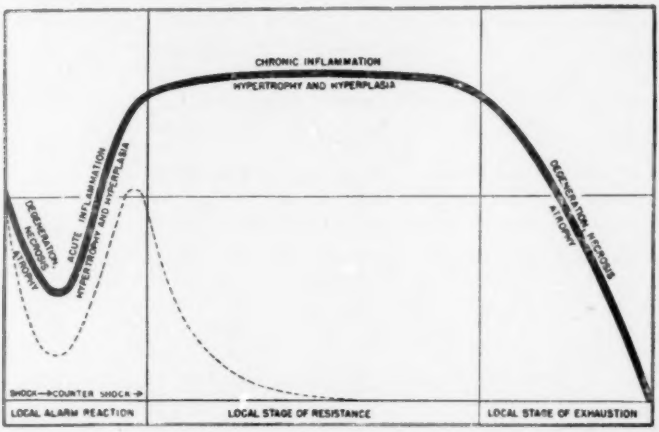

FIG. 1. — *Schematic drawing illustrating the characteristic changes in morphologic structure and topical resistance, as they occur during the three stages of the L-A-S.* — The heavy line represents "specific resistance", the interrupted line, "crossed resistance". (From Selye, in G. Jasmin and A. Robert: "Symposium on the «Mechanism of Inflammation»", 1953, *Acta Inc., Med. Publ.*, Montreal).

In studies concerning systemic stress, it had been found that the beneficial effect of corticoids is most evident during the counter-shock phase of the alarm reaction, at the time when adaptation is being acquired. Thus, for instance, animals have very little resistance to such stressors as forced muscular exercise, cold or drugs, if they are first exposed to them soon after adrenalectomy, unless corticoids are administered in doses far higher than those necessary to maintain them under resting conditions. On the other hand, if animals are previously adapted to these same stressors (and thus led into the stage of resistance) before adrenalectomy, they exhibit considerable resistance to muscular exercise, cold or drugs, even after ablation of their adrenals and without corticoid treatment. It was thought that, during the stage of resistance, the peripheral cells become comparatively independent of corticoids in their defensive functions (2).

It is also a well known fact that immediately after eliciting a general alarm reaction, it is rather difficult to elicit the same systemic response again. Even continuous exposure to increasing doses of a stressor, usually fails in maintaining the typical manifestations of the systemic alarm

reaction (adreno-cortical degranulation, gastric ulcers, hemo-concentration, etc.). If the stressors are very severe, death will ensue, and, if they are not of lethal intensity, the stage of resistance develops, despite continued exposure.

Using the recently described "granuloma pouch" technique for the study of local stress, we wished to establish whether antiphlogistic hormones or a previous local alarm reaction, can also desensitize the tissues to topical manifestations of stress. At the same time, we wanted to determine whether there is a certain "critical period", during the countershock phase of the local alarm reaction, during which this topical desensitization is especially effective.

Experimental material and techniques

Experimental animals. — In all the experiments to be reported here, we used female albino Wistar rats, weighing 114-148 gm.

Treatment with antiphlogistic hormone. — Cortisone was employed in the form of microcrystals of its acetate. It was given in preference to cortisol (hydrocortisone) acetate, because of its more rapid absorption rate. In all the six groups of the first experimental series, in which this preparation was employed, we invariably administered a single dose of 10 mg, suspended in 5 ml of Merck's suspension fluid. This comparatively large volume of suspension was introduced subcutaneously, into the inguinal region. It was then dispersed over a very large territory by massage. The reason for this procedure was that, through the spreading of the hormone microcrystals, absorption was greatly enhanced and the antiphlogistic effect of cortisone could thus be concentrated during a relatively brief period. This was considered essential, as the experiment was designed to explore whether there is a "critical period" of greatest cortisone sensitivity.

The "granuloma pouch" technique. — This has been described, together with its principal applications, in the study of wound-healing and inflammation, in several earlier publications ([6, 7]). For the purpose of the experiments under consideration, it was performed as follows:

After removing the hair from the back of the rat, with fine electric clippers, 25 ml of air is injected into the loose connective tissue of the back, under the skin, between the shoulder blades. A regular ellipsoid air-space is thus created. Then, without removing the needle, the syringe is detached and, through a smaller second syringe, a dilute solution of croton oil is injected into the cavity. In the first experiment, 0.5 ml of an 0.25 % solution of croton oil (in corn oil) was employed, while, in the second experiment, the concentration of the irritant was varied, as described in the text, below. Under the influence of the croton oil, the connective tissue lining the air-space is gradually transformed into a regularly shaped ellipsoid granulona, which produces a hemorrhagic exudate. Normally, the latter gradually replaces the air, until (after about two weeks) the whole pouch is filled by exudate. One of the principal advantages of this technique is that most of the factors which affect inflammation (position, size and shape of the affected focus, etc.), can

be maintained rigidly identical, even if very different kinds of irritants are used, or if the inflammatory reaction is artificially intensified or suppressed.

RESULTS

First Experiment. - The "Critical Period" of Sensitivity to Cortisone. — In the first experimental series, 70 rats were subdivided into seven groups of ten animals each. Group I acted as controls, and received

TABLE I

"Critical period" of sensitivity to cortisone

Group	Day of cortisone treatment *	Exudate (in ml)
I	Control	7.0 ± 2.9
II	— 2	6.7 ± 1.6
III	0	3.3 ± 1.4
IV	+ 3	0.6 ± 0.06
V	+ 6	5.9 ± 2.0
VI	+ 9	10.7 ± 2.1
VII	+ 12	9.3 ± 2.3

* Expressed in relation to the creation of the pouch, which is considered to have been done on 0 day.

no cortisone. However, systemic stress is known to exert a marked inhibitory effect upon the development of the granuloma pouch, and we gave the cortisone in a large volume (5 ml) of the vehicle, which could cause stress. Hence, we injected these controls in the same manner, with 5 ml of suspending agent (without cortisone), massaging the fluid, just as in the hormone-treated groups. In these controls, the injection of the vehicle was performed on the third day, after producing the pouch, because preliminary experiments had shown that, at this time, suppression of inflammation is most readily effected. Thus, in the strictest sense, the control Group I is most comparable with the experimental Group IV, which, as we shall see, did, in fact, manifest the most severe suppression.

The rats of all other groups were given a single subcutaneous injection of cortisone, as indicated above. This was administered at varying time intervals, as outlined in Table I.

It will be recalled that, in this series, we used what has become the "standard technique" for the production of the granuloma pouch (25 ml of air, plus 0.5 ml of an 0.25 % croton oil solution).

All animals were killed on the twentieth day, that is, 18 days after creation of the pouch. Then, the exudate was carefully aspirated, through a needle, into a syringe, and measured. The mean volumes of exudate are listed in Table I (together with the standard error for each group).

It will be noted that our experimental arrangement was such that, both the treatment with cortisone, and the topical irritation conducive

to inflammation, were identical in all experimental groups. The only variable was the time-interval between the administration of the hormone and the introduction of the irritant. Perusal of our data indicates that, by far, the most effective suppression of exudation was obtained in Group IV, in which the cortisone was given three days before the croton oil. Thus, it is clear that, under these conditions, there is a sharply limited "critical period", during which treatment with an antiphlogistic hormone is most effective in suppressing the L-A-S, as judged by its salient morphologic feature, the formation of exudate. It is noteworthy that, in this experimental arrangement, in which croton oil acts as an irritant, exudation begins just about at that same time. In the granuloma pouch technique, this can readily be determined, without injuring the animal, by simple trans-illumination of the pouch, with an electric flashlight. The efficacy of the hormone becomes gradually less pronounced, as we deviate from this "critical period", in either direction.

Numerous other experiments —not published here in detail— had revealed that, if a granuloma pouch is produced by the standard procedure, in animals not otherwise treated (either by hormone or by vehicle), the average amount of exudate, accumulated by the eighteenth day, is quite regularly between 10 and 14 ml. Hence, our controls, Group I, also showed some degree of suppression. This we are tempted to attribute to the systemic stressor effect of injecting 5 ml of the vehicle and massaging it to disperse the fluid over a large area.

Second Experiment. - The Induction of Local Resistance to Chronic Exudative Inflammation by Pretreatment with a Topical Stressor.

In our second experimental series, we wanted to examine the "critical period" in the response to local stress, with an entirely different technique. Here, two groups of ten rats each were given 25 ml. of air, and two doses of 0.5 ml of dilute croton oil; one on the first, the other, on the tenth day. In the first group, an 0.05 % solution was injected on the

TABLE II

Suppression of exudate formation by pretreatment with a topical stressor

Group	Croton oil		Exudate (in ml)
	First day	Tenth day	
I	0.05 %	0.5 %	2.1 ± 0.9
II	0.5 %	0.05 %	16.2 ± 2.6

first day, and 0.5 % on the tenth day. In the second group, the reverse procedure was used, 0.5 % being administered on the first, and 0.05 % on the tenth day. Since preliminary experiments had shown that 0.5 ml of the 0.05 % croton oil produces little or no exudate, it was assumed that the first injection in Group I, and the second injection, in Group II, would not have produced any significant amount of exudate in itself. We wanted to determine now whether such a sub-threshold degree of

topical stress could nevertheless considerably influence the final outcome if administered ten days prior to a fully effective dose (0.5 ml of 0.5 % croton oil).

Perusal of Table II clearly indicates that pretreatment with a threshold dose of croton oil (Group I) inhibits the formation of exudate by the subsequent administration of an otherwise fully effective amount of this same irritant. On the other hand, if the fully effective dose is given first, subsequent treatment with the threshold dose results in no significant suppression.

SUMMARY AND CONCLUSION

Using the "granuloma pouch" technique as an assay method, it is established that cortisone best inhibits exudate formation during a "critical period", which is at its maximum at about the third day after exposure to a topical stressor (croton oil).

It is also shown that pretreatment of a circumscribed area, with a threshold dose of an irritant, so alters topical tissue reactivity that subsequent treatment with an otherwise fully effective dose of the same irritant no longer results in normal exudate formation.

The general implications of these experiments are discused in relation to the similarity of the reaction-patterns observed after exposure to systemic stress on the one hand, and local stress on the other.

These investigations were performed with the aid of a Consolidated Grant of the National Research Council of Canada.

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ATMUNGSGRÖSSE UND BLUT/HIRN-SCHRANKE

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SEITDEM Pflüger (1) im Jahre 1868 als erster die Wirkung von Änderungen der Zusammensetzung der Atmungsluft auf die Grösse der Lungendurchlüftung genauer untersuchte, ist in unzähligen Arbeiten der Zusammenhang zwischen der Beschaffenheit des arteriellen Blutes und der Atmungsgrösse studiert worden. Denn wenn man nicht annehmen will, dass die Zusammensetzung der Luft reflektorisch von der Lungenwand aus auf die Atmungscentren zu wirken vermag —eine Ansicht, die ja gleichfalls vertreten wurde (vgl. Pi-Suñer (2)), aber keine Anerkennung gefunden hat— so liegt es auf der Hand, dass die Atmungsluft nur auf dem Wege über das arterielle Blut die Centren beeinflussen kann. Es hat auch schon frühzeitig nicht an Versuchen gefehlt, einen einheitlichen Faktor in der Zusammensetzung des Blutes zu finden der alle Atmungswirkungen erklären sollte. Bald wurde der Sauerstoff—, bald der Kohlensäuregehalt verantwortlich gemacht, und Winterstein (3) glaubte in der cH des Blutes den einheitlichen Faktor gefunden zu haben. Aber bald musste er selbst dies als irrig erkennen, da er feststellte (4), das bei O_2-Mangel die Reaktion des Blutes sich nicht nach der saueren, sondern gerade umgekehrt nach der alkalischen Seite verschiebt, die Atmungsgrösse in diesem Falle also nicht der cH des Blutes parallel geht, sondern sich gerade entgegengesetzt ändert, offenbar weil die cH-Änderungen des Blutes unter diesen Bedingungen jenen der Atmungsgrösse nicht vorangehen, sondern ihnen nachfolgen. Obwohl so zum ersten Male erwiesen war, dass die Änderungen der Lungendurchlüftung nicht solchen der Beschaffenheit des arteriellen Blutes zu entsprechen brauchen, und später noch zahlreiche analoge Beobachtungen gemacht wurden (vgl. die zusammenfassenden Darstellungen von Gesell (5) und neuere Arbeiten von Gollwitzer-Meier (6) hat man nicht aufgehört, nach gesetzlichen Beziehungen zwischen diesen beiden Grössen zu suchen. Den Gipfel dieser Bemühungen hat wohl Gray (7) mit seiner "multiple factor theory" erreicht, in der er für die Abhängigkeit der Atmungsgrösse von O_2-Druck, CO_2-Druck und der cH des arteriellen Blutes auf statistischer Grundlage Gleichungen zu errechnen versuchte. Zwar haben manche Autoren gezeigt

(vgl. Opitz und Schneider (8), Loeschcke (9)), dass sich vielfach engere Beziehungen der Atmungsgrösse zu der Beschaffenheit des *venösen* als der des arteriellen Blutes ergeben; da aber das venöse Blut von den Centren kommt, und die Änderungen der Atmungstätigkeit jenen der Beschaffenheit des venösen Blutes vorangehen und nicht nachfolgen, können diese letzteren, selbst wenn sich eine gesetzmässige Korrelation ergeben sollte, offenbar nicht als Ursache, sondern nur als Folge der ersteren gedeutet werden.

Nun stehen die Centren nicht nur mit dem Blute in Kontakt, sondern auch mit der Cerebrospinal-Flüssigkeit (CSF), welche die Hirnhöhlungen, die Subarachnoidealräume und die bis tief in die Hirnsubstanz sich fortzetzenden Virchow-Robinschen Räume erfüllt. Dass die Zusammensetzung der CSF sich von jener des (durch die Blut/Liquor-Schranke von ihr getrennten) Blutes in mancherlei Hinsicht unterscheidet, ist echon lange bekannt. Uns interessiert hier im wesentlichen die cH als der die Atmungscentren vor allem beeinflussende Faktor. Die Reaction des Liquors ist nicht, wie zahlreiche ältere Angaben besagen, alkalischer als die des Blutes, sie ist auch nicht, wie noch in neuesten Arbeiten zu lesen ist, gleich der des Blutes, der Liquor ist vielmehr *normalerweise stets weniger alkalisch* als das Blut (Winterstein und Gökhan (10)). Noch wichtiger aber ist, dass dieser unter gewöhnlichen Bedingungen (beim Hunde) meist zwischen pH 0.1 und 0.2 liegende Unterschied nicht konstant ist. ja sich nicht einmal stets in beiden Flüssigkeiten in gleichem Sinne ändert. Diese Tatsache ist zuerst von Gesell und Hertzman (11) festgestellt worden, die unter dem Einfluss einer intravenösen Injection von Bikarbonat eine gegensinnige pH-Änderung in Blut und Liquor beobachteten. Das Blut wurde alkalischer, der Liquor sauerer. Da unter diesen Bedingungen die Atmungstätigkeit *zu-* und nicht abnimmt, ergibt sich daraus die wichtige Erkenntnis, dass für die Änderungen der Atmungsgrösse pH-Verschiebungen in der CSF von grösserer Bedeutung sind als solche im arteriellen Blut. Viel weitgehender sind nun die Schlussfolgerungen, die sich aus den Versuchen von Winterstein und Gökhan (10) ziehen lassen. Diese untersuchten die sogenannte Ammoniumchlorid-Acidose. Es ist schon lange bekannt, dass die Einführung von Salmiak eine Verschiebung der Blutreaction nach der saueren Seite bewirkt. Die Zunahme der Lungenventilation entspricht nun keineswegs dieser im Verhältnis zu anderen sauren Substanzen ungewöhnlich starken cH-Vergrösserung. Die Ursache dieses Verhaltens wurde darin gefunden, dass auch hier eine gegensinnige Änderung des pH in Blut und Liquor eintritt, und dieser letztere eine Verschiebung nach der *alkalischen* Seite erfährt, die offenhar der durch die Hyperhydrie des Blutes bedingten Atmungsverstärkung entgegenwirk. Die Atmungsänderung its also das Resultat der algebraischen Summe der entgegengesetzt wirkenden Reaktionsäderungen. Während nun bei normalen Hunden die atmungsverstärkende Wirkung der Blutsäuerung die atmungsabschwächende Wirkung der Liquoralkalinisierung überwiegt, tritt nach Ausschaltung der Chemoreceptoren das gerade Gegenteil ein: Jetzt ist nach Zufuhr von NH_4Cl trotz der Verschiebung der Blutreaction nach der saueren Seite die Atmungsgrösse bedeutend herabgezetzt. Daraus ergibt sich die wichtige Schlussfolgerung,

dass die cH des Blutes auf die Atmungscentren *nur reflektorisch durch die Chemoreceptoren wirkt* und nach deren Ausschaltung keinen direkten Einfluss mehr auf sie ausübt. Der direkte Einfluss geht vielmehr von der CSF, bezw. der in ihrer Zusammensetzung ihr offenbar entsprechenden intercellularen Hirnflüssigkeit aus, die durch die Blut/Liquor/Hirnschranke vom Blute getrennt, die in diesem stattfindenden cH-Änderungen keineswegs in gleichem Ausmasse oder auch nur in gleichem Sinne zu erfahren braucht.

So erklärt sich vom Standpunkte der "Reactionstheorie" (zusammenfassende Darstellung bei Winterstein (12)) das ihr scheinbar widersprechende Verhalten der Lungendurchlüftung bei Bikarbonatinjection und in der NH_4Cl-Acidose in einfacher Weise: Die Blut/Liquor-Schranke ist für H^+ bezw. OH^- Ionen schwer, für wasser- und lipoidlösliche elektroneutrale Verbindungen dagegen leicht durchlässig. Wird HCO_3^- in die Blutbahn gebracht, so wird bei seiner Dissociation ($HCO_3^- \rightleftarrows CO_2 + OH^-$ das Blut alkalischer, gleichzeitig aber passiert das hierbei entstehende elektroneutrale Kohlendioxyd leicht die Blut/Liquor-Schranke und erzeugt auf der anderen Seite derselben gemäss der Gleichung $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+$ eine atmungsverstärkende Reaktionsverschiebung nach der *saueren* Seite. Als getreues Gegenstück dazu wird bei Einführung von NH_4^+ in die Blutbahn bei seiner Dissociation ($NH_4^+ \rightleftarrows NH_3 + H^+$) im Blut die Reaction nach der saueren Seite verschoben, während das elektrisch neutrale Ammoniak durch die Blut/Liquor-Schranke hindurchtretend auf deren anderer Seite enstprechend der Gleichung $NH_3 + H_2O \rightleftarrows NH_4 + OH^-$ die atmungsabschwaächende Reaktionsverschiebung der CSF nach der *alkalischen* Seite bewirkt.

Noch zwei weitere Schlussfolgerungen lassen sich aus diesem Verhalten ziehen. Erstens: der Nachweis, dass die Blut-Liquor-Schranke eine Barrière gegenüber den im Blute eintretenden Reactionsänderungen darstellt, beweist aufs Neue, dass der die Atmungstätigkeit bestimmende Faktor die H-Ionen sind und nicht das CO_2, gegen das es wegen seiner grossen Wasser- und Lipoidlöslichkeit keine Barrière geben kann. Zweitens: wenn Blut/Liquor und Blut/Hirn-Schranke nicht identisch sind, indem entsprechend der älteren Auffassung der Liquor selbst überall zwischen Blut und Hirnzellen zwischengeschaltet ist, so müssen doch beide Schranken zum mindesten in ihrem Verhalten gegenüber H-Ionen mit einander übereinstimmen, da die Änderungen der Lungendurchlüftung jenen der Liquor-Zusammensetzung parallel gehen. Diese letzteren, die wir als *liquorogen* bezeichnen wollen, können mithin als Mass der in der intercellularen Flüssigkeit oder in den Hirnzellen selbst stattfindenden Veränderungen dienen.

Bikarbonat-Alkalose und Salmiak-Acidose sind besonders charakteristische Beispiele, die in —man könnte sagen übertriebener Weise— die Unabhängigkeit der Atmungstätigkeit von der Beschaffenheit des arteriellen Blutes dartun. Im allgemeinen wird man erwarten dürfen, dass die pH-Änderungen in Blut und Liquor gleichsinnig erfolgen. Damit ist aber nicht gesagt, dass sie gleichzeitig und in gleichem Ausmasse vor sich gehen. Dies ist angesichts der von uns aufgezeigten Barrièrewirkung nicht einmal wahrscheinlich. Damit dürften sich vielleicht zahlreiche vom

Standpunkte der bisherigen Theorien kaum verständliche Erscheinungen einer Erklärung zuführen lassen. So erzeugt z. B. nach Ausschaltung der Chemoreceptoren O_2-Mangel bekanntlich keine Verstärkung, sondern gerade umgekehrt eine Abschwächung der Atmung. Dies ist mitunter auch nach Wiederherstellung der Luftatmung zu beobachten (Gollwitzer-Meier ([6])). Die Blutbeschaffenheit gibt keinerlei Erklärung für dieses Verhalten. Untersucht man aber die hierbei in der CSF eintretenden Veränderungen (Gökhan und Winterstein, Hoppe-Seylers Ztschr., 1953, im Druck), so findet man, dass bei O_2-Mangel sowohl bei normalen wie bei Chemoreceptorenlosen Tieren, bei spontaner wie bei konstanter künstlicher Atmung der Liquor alkalischer wird und diese Alkalescenz auch nach Wiederherstellung der Luftatmung noch beibehält.

Auch noch auf andere Weise lässt sich die Bedeutung der Blut/Liquorschranke für die Lungendurchlüftung demonstrieren. Schon lange ist bekannt, dass es eine Anzahl Pharmaka gibt, die ihre atmungserregende Wirkung nur reflektorisch auf dem Wege der Chemoreceptoren ausüben und nach deren Ausschaltung wirkungslos bleiben. Das gilt nicht nur für das Salmiak, dessen auf cH-Steigerung beruhende atmungerregende Wirkung nach Entfernung de Chemoreceptoren, wie wir oben gehört haben, verschwindet, das gilt in gleicher Weise für Lobelin und für Natriumcyanat. Alle diese Mittel werden wieder wirksam, und zwar in unvergleichlich geringerer Dosis, wenn man sie unter Umgehung der Blut/Liquor-Schranke durch suboccipitale Injection direkt in die CSF einführt (Winterstein und Gökhan ([13])).

So können wir zusammenfassend sagen, dass *die Beschaffenheit des arteriellen Blutes keine direkte Wirkung auf die Atmungscentren ausübt, sondern die Lungendurchlüftung nur indirekt beeinflusst, entweder reflektorisch durch die Chemoreceptoren oder auf dem Wege der Cerebrospinal-Flüssigkeit, deren Veränderungen durch die Blut/Liquor-Schranke entscheidend mitbestimmt werden.*

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THE LAW OF FOLLICULAR CONSTANCY

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MANY fields of endocrinology have been sign-posted by Professor Alexander Lipschutz, and almost every chapter in our knowledge of the physiology of reproduction bears the mark of his hand. We owe to him some of our basic information about the behaviour of gonadal grafts, and our present knowledge that ovarian tissue can withstand deep freezing to —79 and even —190° C., and then be successfully grafted (Parkes & Smith, 1953), seems less remarkable when we remember that Lipschutz had found as long ago as 1929 and 1930 that desiccated ovarian tissue that has lost 50 % of its original weight will still "take". His researches into the reaction of an ovary after the removal of its fellow, or of a fragment of an ovary after the removal of the rest of the ovarian tissue of the body, led to conclusions which have been summed up in the "Law of Follicular Constancy" (Lipschutz, 1925, 1928; Lipschutz & Voss, 1925). His studies of the changes which occur in the accessory reproductive organs and mammary glands, when only a fragment of ovarian tissue is left in the body, revealed much that was unknown before about ovarian-pituitary relationships (e. g. Lipschutz and Vinals, 1934; Lipschutz, 1937-8). As he was able to show, the gonadotrophic function of the pars distalis of the pituitary is in equilibrium when both ovaries are present in the body. When, however, only a fragment of ovary remains, the quantitative relations are so disturbed that the gonadotrophic powers of the pituitary are no longer inhibited... "le fragment ovarien seul est incapable de reprendre le freinage de la préhypoyhyse". His study of the aberrant changes which occur in these conditions immediately led to his now classical researches on the tumorigenic action of certain steroids and the opposing actions of others.

In this short article, I propose to confine myself to some observations that relate to Lipschutz's "Law of Follicular Constancy". This Law states that a given number of follicles mature in the ovaries during each oestrous cycle, regardless of the amount of ovarian tissue in the body. The stimulus to maturation is more or less constant, and is provided by

the gonadotrophic hormone of the pituitary. The compensatory hypertrophy which either a single ovary or a fragment of ovarian tissue undergoes after the removal of its fellow (or of part of the second ovary as well), is due to the maturation of more follicles than would normally grow, and to their subsequent luteinization. The smaller the ovarian fragment left in the body, the sooner will recurrent phases of follicular maturation denude it of its oocytes.

Implicit in this general conception of ovarian dynamics, which we owe primarily to Lipschutz, is the belief that neoformation of oocytes does not take place in the adult ovary.

Over the period when Lipschutz was conducting the experiments that led to the formation of this "law", other workers were making observations which appeared to lead to a contrary view that "oogenesis can continue after puberty, and that it occurs as a result of a cyclical proliferation of the germinal epithelium in phase with the oestrous cycle" (e. g. Allen, 1923; Evans & Swezy, 1931). In the belief that this was the correct view, certain experiments were planned in my own laboratory; but the results were different from those anticipated in so far as they were not consonant with the idea that oogenesis went on in the mature animal. I then undertook a general study of the whole question, and reviewed the different kinds of evidence that had been advanced in favour of the two views (Zuckerman, 1951). After considering such matters as the technique of counting oocytes; the influence of age on oocyte numbers; variation in the numbers of oocytes during the oestrous cycle; cyclical variations in the rate of proliferation of the germinal epithelium; the reaction of the germinal epithelium to vital staining; variations in the numbers of oocytes in ovarian grafts; the influence of destruction of the germinal epithelium on numbers of oocytes; the effects of X-irradiation on the ovary; the regeneration of ovarian tissue *in vivo*, and its cultivation *in vitro;* the phenomenon of compensatory hypertrophy in the normal and X-irradiated ovary; as well as the cytological evidence relating to the occurrence of meiosis: it became clear that, with the exception of a few species (e. g. the lemur and armadillo), there is no real evidence which proves that the mammalian ovary continues to produce oocytes after puberty.

Since then, further experiments on the subject have been carried out in my laboratory; their results also accord fully with the view that oogenesis normally ceases before puberty.

1. Variation in the numbers of oocytes during the menstrual cycle.

All investigators who have studied the ovary have been impressed by an apparent increase in the number of follicles at about the time of ovulation. It was actually this impression — and not counts of the total number of oocytes in the ovary at different phases of the cycle — which helped to encourage the view that oogenesis continues in the adult animal and in phase with the oestrous cycle. The impression loses substance on close view. A detailed analysis of the variations which occur in the total numbers of oocytes in the different phases of the oestrous cycle of the

rat (Mandl & Zuckerman, 1950) showed that there were no statistically significant differences in the total number of oocytes present in early oestrus, oestrus, late oestrus, early dioestrus, dioestrus, or late dioestrus. On the other hand, there is no doubt that cyclical changes do take place in the number of medium and large follicles in the adult ovary (e. g. Mandl & Zuckerman, 1952). In the course of these studies we (Mandl & Zuckerman, 1951b) confirmed earlier findings (Arai, 1920a) that the total number of oocytes in the ovaries of the rat decreases with age, and found that the variance of the total number of follicles is greater between unrelated rats of different age than it is between litter-mates.

A similar study of fourteen mature rhesus monkeys (Green & Zuckerman, 1951), also failed to reveal significant differences between the average total number of oocytes present at the beginning, the middle or the end of the menstrual cycle. This study has since been extended by making total oocyte counts of the ovaries of forty-two sexually-mature rhesus monkeys, including the twelve previously studied (Green & Zuckerman, unpublished). The data again fail to reveal any significant differences in total oocyte numbers between the early, middle and late phases of the menstrual cycle. Unlike our study of the rat, this investigation of the monkey had the shortcoming —which it will probably be impossible to overcome— that no account could be taken of litter relationships or the ages of the animals studied.

2. The number of oocytes in ovarian fragments

Although compensatory ovarian hypertrophy may result in a three-times gain in weight, the total number of oocytes in the hypertrophied ovary is no greater than what would be expected in a single normal ovary. In fact, it may be less (Arai, 1920b; Mandl & Zuckerman, 1951a). In experiments on rabbits and cats from which they removed the whole of one ovary and part of the second, Lipschutz (1925, 1928) and Lipschutz & Voss (1925), long ago observed that the total number of primary follicles present "was greatly reduced as compared with ovarian fragments present in normal females".

This conclusion has now been further corroborated in a new set of experiments on rats (Mandl, Zuckerman & Patterson, 1952). Not only has it been found that the number of oocytes in a hypertrophied fragment of ovary do not increase in number; it has also been shown that the rate of loss of oocytes after unilateral ovariectomy is substantially greater than would be expected in a single ovary of a normal animal. The rate appears to increase inversely with the amount of ovarian tissues left in the body.

3. Effects of X-irradiation of the ovary

In spite of certain statements that have been made to the contrary, it appears certain that if X-ray sterilization is complete, and all primordial and other follicles are destroyed, the ovaries remain permanently sterile (Lacassagne, 1913; Desaive, 1940). Recent experiments of our

own on rabbits and mice are fully in keeping with this conclusion (Humphreys & Zuckerman, 1953). They also show that when irradiation is complete, in the sense that all oocytes and follicles are destroyed, compensatory hypertrophy cannot occur. There was no exception to this rule in seventeen animals (ten mice and seven rabbits); whereas, when hypertrophy did occur, histological study showed that the ovaries still contained follicles and oocytes. This finding, which helps to resolve an unsettled point in the story of compensatory hypertrophy, can be regarded as being in keeping with Lipschutz's (1930) suggestion that the endocrine activity of ovarian tissue depends upon the presence of ova capable of follicular development. When sterilization destroys all follicular cells, ovarian tissue appears to be incapable of producing oestrogen.

4. The effect of hypophysectomy on the number of oocytes

Swezy (1933) has suggested that a variety of hormonal influences can affect the rate of oogenesis in mature rats. In particular her observations seemed to show that the total number of oocytes is greater in hypophysectomized than in normal rats, and she therefore inferred that the presence of the anterior pituitary inhibits oogenesis. This conclusion, which presupposes that oogenesis occurs in the mature rat, was based upon an estimation of the number of oocytes in one of the ovaries of each of five normal and eight hypophysectomized animals. Since it is not the only conclusion that could be drawn from the figures which were obtained, the experiment was repeated in my own laboratory by Ingram (1953), using fourteen pairs of litter-mate rats, each pair consisting of one hypophysectomized and one normal animal. The total number of oocytes proved to be greater in nine, and less in five, of the hypophysectomized-normal comparisons. This ratio is not significantly different from one of 7:7. The experiment thus provided no evidence in support of the belief that neogenesis of oocytes occurs after hypophysectomy, or that it continues at an enhanced rate. Analysis of the figures did, however, suggest that the rate at which oocytes disappear from the ovary may be slower after hypophysectomy than in normal conditions due, presumably, to the fact that maturation of follicles ceases to occur in the absence of a gonadotrophic stimulus.

Conclusion

The results of these newer experiments, like those which I considered in my previous review (Zuckerman, 1951), thus conform to a main presupposition of Lipschutz's Law of Follicular Constancy, in so far as they indicate that oogenesis does not occur in the ovary of the adult rat, the adult rabbit, or the adult monkey. Certain recently published observations by other workers could, however, be thought to conflict with this conclusion. Thus Aron, Marescaux & Petrovitch (1952) report findings which suggest that the guinea-pig should be classified with the lemur and armadillo as exceptions to the general rule that oogenesis does not occur after puberty. It has also been suggested that fragments of mature ovarian tissue of the rat are able to regenerate oocytes after deep freezing and the disappearance of all oocytes (Parkes & Smith,

1953). The number of oocytes present in the grafts studied in these experiments were, however, very small, and it may well be that such oocytes as appear in grafts of mature ovarian tissue after deep freezing indicate the persistence of cells which were not completely destroyed by the treatment. This observation applies only to grafts of mature ovarian tissue. Oogenesis continues in most mammals up to some time before puberty. Simpson & van Wagenen (1953) have recently shown that purified sheep FSH injected into prepubertal monkeys stimulates growth of follicles of all sizes. These workers report that the total follicle counts were not changed as a result of the treatment, but that the oocyte content of the stimulated ovaries was increased because of the multiplication of polyovular follicles. It is by no means unlikely, therefore, that oocytes would continue to form in grafts of deep-frozen prepubertal ovaries. Several considerations indicate that a comparison of the pre- and post-pubertal ovary in such conditions as compensatory hypertrophy and grafting will yield much interesting information. It is unsafe to make predictions about experiments of this kind: but on the face of it it seems highly improbable that the results will fail to conform with one of the few Laws we have in Endocrinology — Lipschutz's Law of Follicular Constancy.

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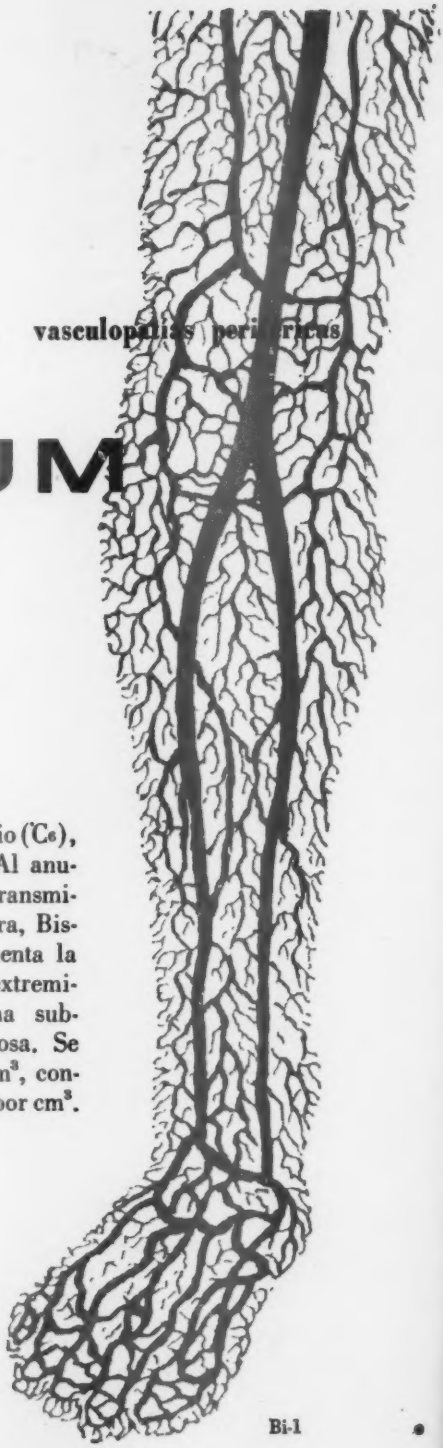

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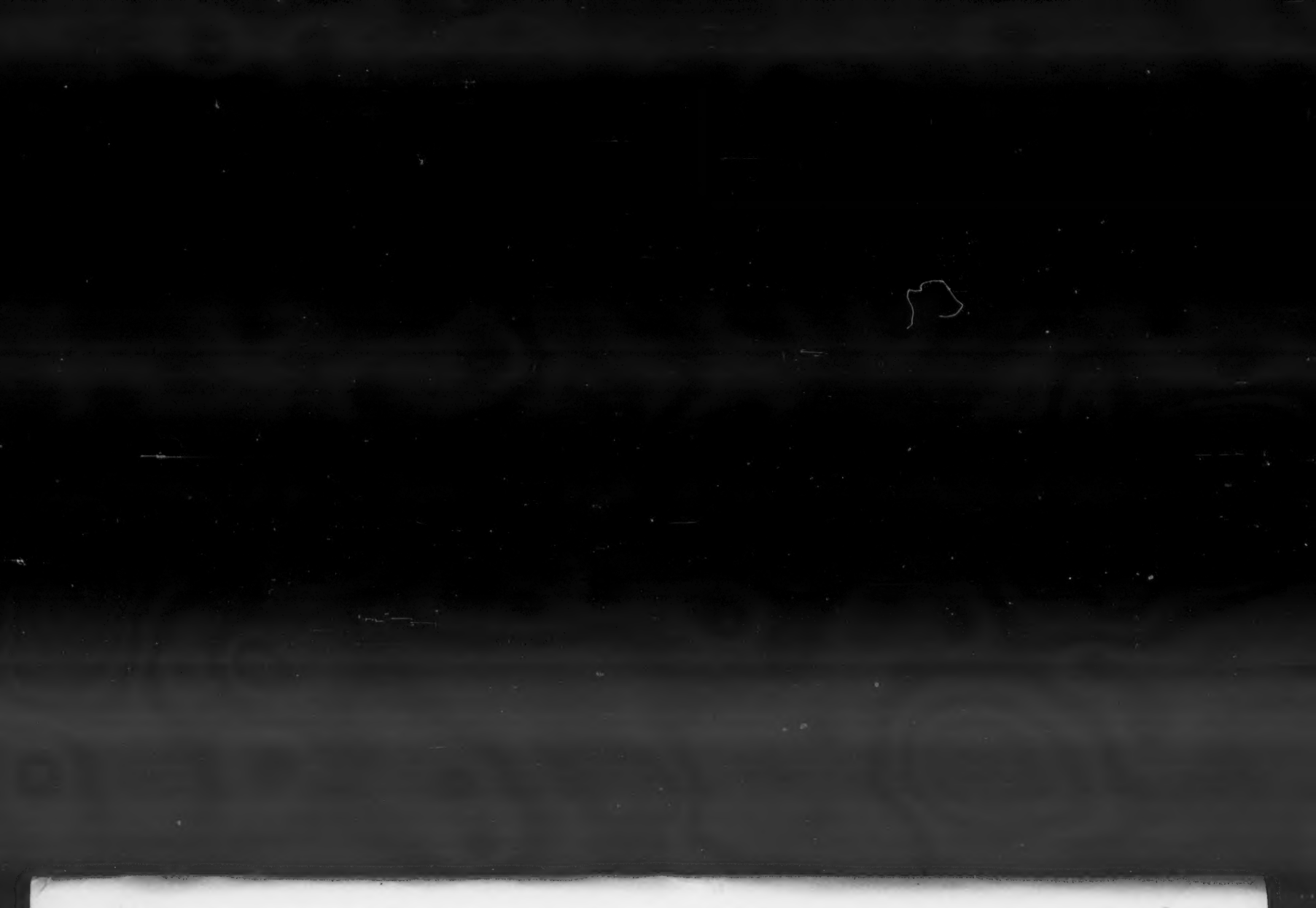

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milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Angstrŏm	A	miligramo	mg	segundo	s
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